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BIOTRIBOCHEMISTRY OF THE LUBRICATION OF NATURAL JOINTS

BIOTRIBOCHEMIA SMAROWANIA NATURALNYCH STAWÓW

Key words:

biohydrophobicity, wettability, interfacial energy, friction coefficient and pH

Słowa kluczowe:

biohydrofobowość, zwilżalność, energia powierzchniowa, współczynnik tarcia i pH

Summary

In this communication, we attempt to consider changes occurring in the phospholipid biomembrane surface in terms of acid-base equilibria. Spe-

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cifically, the influence of the pH on surface wettability, interfacial energy, and surface friction is discussed under consideration of a biomembrane as an amphoteric polyelectrolyte. We measured the interfacial energy of phosphatidylserine (PS) using a model of bilayer membrane in aqueous electrolyte over the pH range 2.0-12.0.0. Micro-electrophoresis was used to examine the local acid-base equilibrium of the membrane surface, which can be considered to mimic the interface of natural joints. In our findings, the biohydrophobicity of natural surfaces is greatly reduced in the presence of phospholipids. We were able to demonstrate that the coefficient of friction *vs.* biohydrophobicity of articular cartilage is interface-controlled by osteoarthritis.

INTRODUCTION

The field of biotribochemistry focusses on the chemistry of biomacromolecules and phospho-lipids as additives, and lubrication on natural surfaces under tribological stress. Nature solves its lubrication mechanism, resulting in the formation of a lamellar biotribofilm and for this reason the mechanism of biofilm's growth and removal (wear) remains largely unknown [**L. 1, 2**].

The control of natural surface**'**s chemical and physical parameters appears to be of prime importance in ensuring effective aqueous biolubrication including that in natural joints. Poor biolubrication in natural joints can be attributed to deterioration of biohydrophobicity (wettability declining from 105° to about 60°), interfacial energy changes of articular cartilage (AC) and changes in composition of synovial fluid (SF) [**L. 2–7**].

The biohydrophobic/biohydrophilic surfaces, such as the phospholipid (PLs) membranes, have surfaces that with changed fluid pH can be either positively ($-NH_3^+$) or negatively ($-PO^-$) charged, or be neutral, because phospholipids are amphotheric and weak polyelectrolytes only partially charged at moderate pHs and near their dissociation constants, pK_a [**L. 8**]. Results of investigation of the influence of pH on wettability and friction coefficient of amino- and carboxy-polyelectrolyte multilayered film combinations like poly(acrylic acid) (PAA) and poly-(allylamineH^+Cl^-) (PAH) salt or poly(L-lysine**)** (PLL) and hyaluronic acid (HA) may be useful in tissue engineering, gene carrier technology, and joint lubrication [**L. 9–11**].

In this study, we examined by microelectrophoresis the influence of pH on (*i*) membrane phospholipid surface's charge density and interfacial energy, and (*ii*) influence of wettability on coefficient of friction using a model phospholipid bilayer-membrane. The relationship between surface wettability and friction is greatly reduced and is characterized by suppressed multilamellar layer formation this leading the surfaces in contact to enhanced friction as presented in a graphical form.

EXPERIMENTAL

The instrumentation used for the microelectrophoretic measurements and methods was described in previous papers [**L. 4, 12–14**]. The phospholipids were purchased from Fluka. Standard solutions of pH 2.0 12.0 were prepared using 0.2 M hydrochloric acid and 0.2 M sodium hydroxide in 0.1 M sodium chloride. Interfacial tension was measured of freshly obtained membranes 10 times for each pH electrolyte solution, and three times for the electric surface charge of the liposome membrane. The method used was based on the Young-Laplace-Kelvin equation, $2\gamma = r\Delta p$, where γ is the surface tension, r is the sphere's radius, and Δp is the difference of pressure between inside and outside of the sphere of radius r. The dependence of interfacial energy on the pH of an electrolyte solution has the form [**L. 12**]:

$$\gamma = \gamma_{\max} + 2sRT \ln\left(\sqrt{\frac{K_a}{K_b}} + 1\right) - sRT \ln\left[\left(\frac{K_a}{a_{H^+}} + 1\right)\left(\frac{a_{H^+}}{K_b} + 1\right)\right] \tag{1}$$

where: K_a – and K_b – is the acid and base equilibrium constant, respectively, s – is the surface concentration of PLs [mol/m^2], R – is the gas constant, T – temperature, $\gamma_{\max}$ is the maximum interfacial energy of the lipid membrane [mJm^{-2}], and a_{H^+} – is the activity of proton.

RESULTS AND DISCUSSION

The dependence between interfacial energy of a lipid membrane formed with phosphatidylserine and the pH of the electrolyte solution is presented in **Fig. 1**. A maximum of the interfacial energy ($\gamma_{\max}$) value of the PS membrane was found to be 2.93 mJm^{-2} at a pH of 3.80. The surface en-

ergy (γ) change follows a very characteristic trend, increasing with the charge loss on the amino group ($-NH_3^+ \rightarrow -NH_2$), and decreasing (γ) with the charge gain on the phosphate ($-POH \rightarrow -PO^-$) group, as the solution pH is increased. The extremely low friction coefficient that is seen in natural joint systems at pH ~ 7.4, indicates that the phosphate ($-POH <=> -PO^-$) equilibrium rather than the ($-NH_3^+/-NH_2$) pair is creating such a favorable environment.

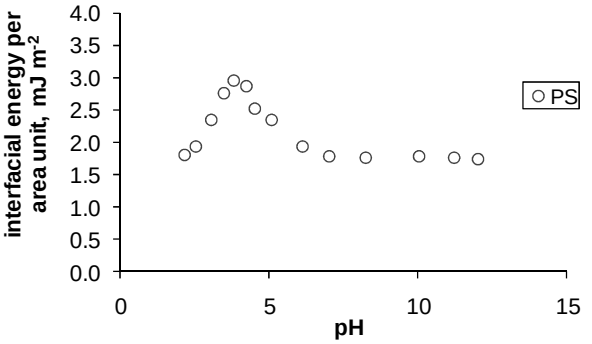

Fig. 1. Plot of interfacial energy of phosphatydylserine (PS) (pK_a (-POH) = 2.42 ; pK_a $(-NH_3)^+$ = 8.02) membrane *vs.* the pH of the electrolyte solution with a maximum surface tension value of 2.93 mJm^{-2} at a pH of 3.80 in a 0.1M KCl solution

Rys. 1. Zależność energii powierzchniowej membrany fosfatydyloseryny (PS) od pH w 0,1 M roztworze KCl

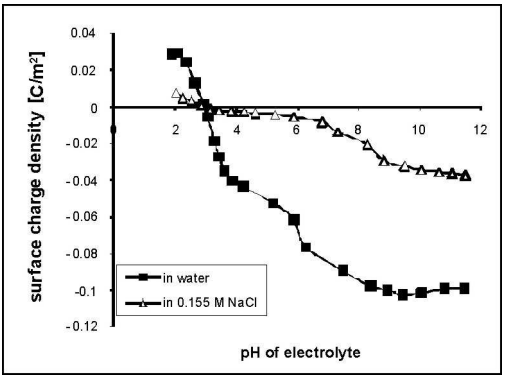

Fig. 2. Plot of the surface charge density of a liposomal membrane formed by phosphatidylcholine in deionized water and a 0.155 M NaCl solution

Rys. 2. Gęstość ładunku membrany fosfatydylocholiny w zależności od pH w wodzie (-■-) i 0,155 M roztworze NaCl (-Δ-)

The pH dependence between the surface charge of the liposomal membrane in a 0.155M NaCl solution and water (control curve) is plotted in Fig. 2. In acidic solution (*i*) we can expect the presence of (-POH) in molecular form, while the [-$N^{(+)}(CH_3)$] groups are adsorbed by Cl^- ions. With increasing pH of solution in the presence of sodium chloride, an increase in the negative charge of the membrane is observed: (*ii*) in basic solution, the [-$N^{(+)}(CH_3)$] groups of the phosphatidylcholine molecules are covered by OH^- ions, whereas the (-PO^-) ones are adsorbed by Na^+ ions.

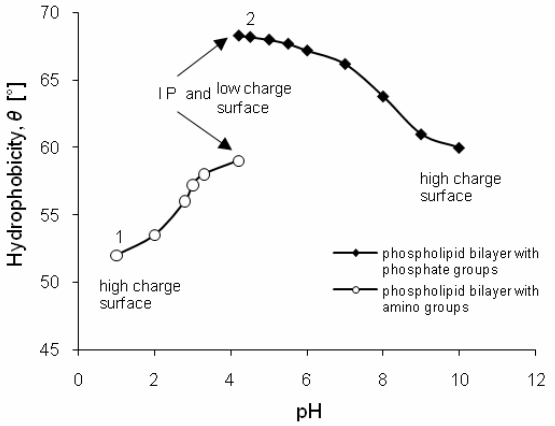

Fig. 3. Influence of solution pH on the hydrophobicity (θ) of the phospholipid bilayer surface with functional groups: (NH_3^+ → $-NH_2$) (curve 1), and (-POH → $-PO^-$) (curve 2), where IP is the isoelelectric point

Rys. 3. Wpływ pH na hydrofobowość dwuwarstwy fosfolipidowej dla grupy aminowej (krzywa 1) i fosforanowej (krzywa 2), IP jest punktem izoelektrycznym

At a low pH, the bilayered PLs is in its protonated form (-NH_3^+) and the wettability is low (curve 1). As the pH of solution is increased, (-NH_3^+) begins to lose its proton (or charge) (-NH_3^+ → -NH_2) and the wettability becomes higher.

Phospholipids can assemble into a micellar (L1) phase, vesicles, bilayers, and anisotropic liquid-crystalline (LLC) phases such as lamellar and hexagonal structures [**L. 1, 15**]. The biolubricant in the synovial joint contains hyaluronate (HA), proteoglycan 4 (PRG4) which is also known as lubricin, and phospholipids (PLs). This mixture is mostly responsible for the ultra-low friction mechanism in the joint [**L. 18**]. On the other hand, the phospholipid molecules that are not involved in the de-

velopment/maintenance of the surface amorphous layer (SAL) associate to form a particular micellar structure (**Fig. 4**) comprising both unilamellar and bilamellar folded spheres [**L. 17, 20**].

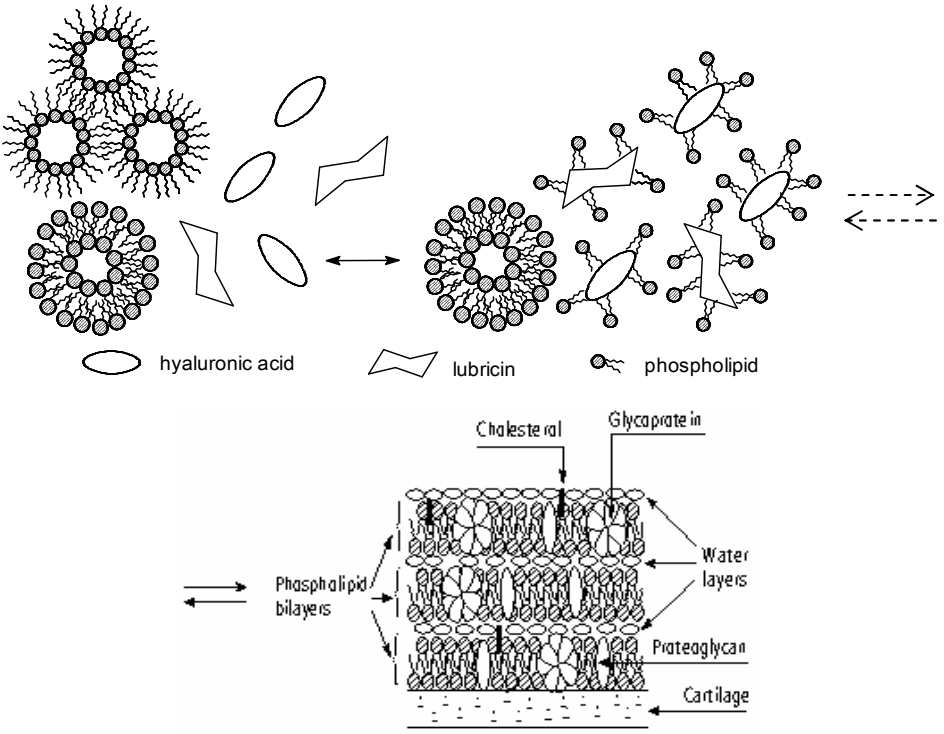

Fig. 4. Equilibrium between macromolecules of lubricin and hyaluronate associated with molecules of phospholipids in synovial fluid on the surface of articular cartilage and assembled into the phospholipids lamellar structure (SAL) [L. 16]. There are two effects which can influence the vesicle morphology during the interaction with hydrophobic surface. These are: (*i*) increase in the vesicle size, and (*ii*) reorganization into a lamellar structure

Rys. 4. Równowaga pomiędzy makromolekułami lubrycyny i hyaluronu z fosfolipidem w płynie synowialnym i tworzenie lamelarnych dwuwarstw na powierzchni chrząstki stawowej

Most of the phospholipids have isoelectric point IP at a pH between 2.9 and 4.5 [**L. 14**]. As the solution pH is increased and the phosphate group looses its proton (curve 2) leaving the biomembrane.

We argue/hypothesize that [**L. 19**] this structure acts in the manner of reverse micelles (supplied by free PLs in synovial fluid) which dissipate energy and thus protect the cartilage from mechanical degradation.

A stiff and flexible form of (HA), which is known to possess a unique water retention capacity and proper viscoelasticity-promoting properties, and hydrophobic interaction with phospholipids, is a component of this lubricant, and is believed to control the pH of synovial fluid and its protein content. It is experimentally proven [**L. 18, 20**] that lubricin and (HA) are the carriers of insoluble PLs molecules and other macromolecules supporting and maintaining the structure responsible for the lubrication as we have shown.

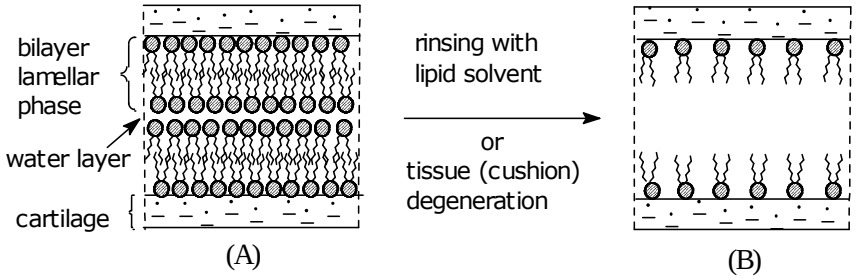

Fig. 5. Schematic illustration of the hydrophilic (HL-HL) model with lamellar layers (A) and hydrophobic (HB-HB) degenerated model (B) of an intact articular cartilage in which articular surface has lost part of its hydrophilic dispensation component. The surface amorphous layer (SAL) is practically lost, leading to the development of the hydrophobic (HB-HB) biopairs [L. 14, 21]

Rys. 5. Model ilustrujący hydrofilowe (HL-HL) lamelarne dwuwarstwy (A) i hydrofobowe (HB-HB) powierzchnie chrząstki stawowej pozbawionej warstw (SAL) (B) [L. 14, 21, 22]

It may be justified to hypothesize that there are two regimes of synovial fluid intervention in joint lubrication. The first is the full-fluid film lubrication (**Fig. 5 A**) that corresponds to frictionless lubrication, which is characterized by complete separation of the surfaces by pressurized synovial fluid. The other (**Fig. 5 B**) corresponds to the boundary lubrication film and is highly complex, involving surface topography changes, physical and biochemical changes of the synovial fluid and articular surface/whole cartilage, and is characterized by an appreciable increase in f. The increase in the coefficient of friction might be associated with a decreasing hydrophobicity of the articular surface (lower amount of PL_{AC}), which may in turn result in the inability of the highly hydrated three-dimensional surface amorphous layer to resist compressive forces during joint loading [**L. 20**].

At this juncture, it is desirable to shed more light onto the phenomenon proposed in a hypothetical mechanism presented in **Fig. 5.** The fundamental premise is that some degree of surface hydrophobicity is required before the hydrophilic layer of phospholipid bilayer that is necessary for lubrication can be created. Consequently, the f of the contacting joint surfaces will increase dramatically with loss of hydrophobicity. This hypothesis or "theoretical" assertion can also be developed from an interpretation of the results by [**L. 2**] in which the relationship between coefficient of friction and hydrophobicity, in terms of contact angle between saline and the articular surface, was presented.

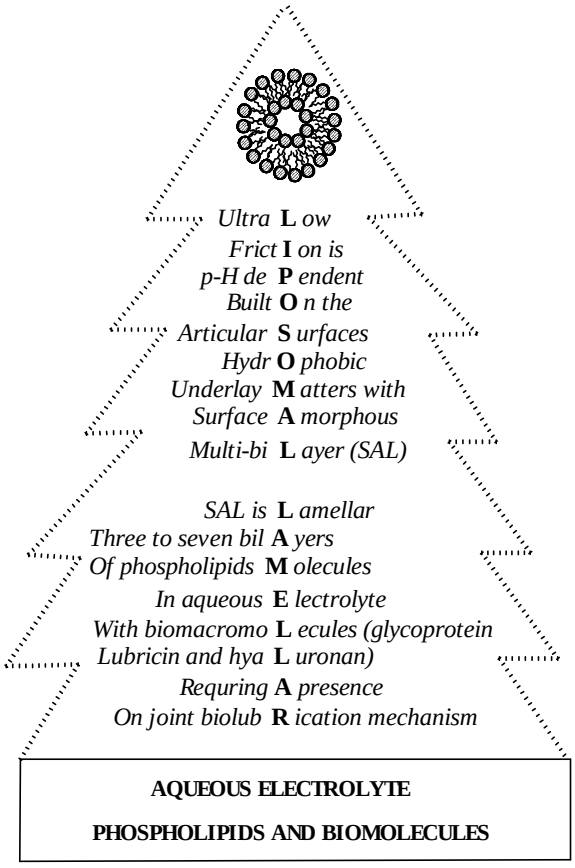

Fig. 6. "Biotribochemical Tree"
Rys. 6. „Drzewo Biotribochemiczne"

A "Biotribochemical Tree" shown in **Fig. 6** summarizes our understanding of some the most important processes occurring between natural surfaces and aqueous lubricant**s** [**L. 23, 24**]. Lubrication in nature is based on liposomes (micelles) in SF and lamellar surface amorphous multilayer (SAL) formed on articular hydrophobic surface. SAL membrane is a warranty of ultra-low friction, and its disappearance is a consequence of a lower PLs concentration in cartilage. When the hydrophobicity of AC drops by natural causes from 105° to about 60° it is loosing its capacity to build SAL of natural surface and the friction increases [**L. 25, 26**].

CONCLUSIONS

It has been speculated that the negatively charged PLs surface of amorphous layer found on the surface of normal intact cartilage is not formed on artificial surfaces. Friction of natural cartilage surfaces was studied by many investigators and a large number of models and theories have been proposed over the years. In this study, biotribochemistry of joint lubrication is based on the Brian Hills idea, who understood very deeply that liposomal (micellar) fluid-lubricant and lamellar nano-layers of phospholipids are a key of natural lubrication. Normal joint has complete separation of the lamellar surfaces by liposomal synovial fluid that corresponds to non-contact surfaces in motion and the ultra-low friction. We showed that acid–base equilibria (pH) taking place in the phospholipid bilayer are crucial for explanation of some biolubrication processes. Biochemical changes in the articular cartilage, as well as the SF, can lead to destroy the AC surface.

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REFERENCES

1. Hills B.A., The Biology of Surfactants. New York: Cambridge University Press; 1988.
2. Hills B.A., Proc Inst Mech Eng Part H: J Eng Med., 214 (2000) 83.

3. Crockett R., Tribology Lett., 35 (2000) 77.
4. Pawlak Z., Figaszewski Z.A., Gadomski A., Urbaniak W. and Oloyede A., Tribol. Intern., 43(2010) 1719.
5. Wierzcholski K., Miszczak A., Tribologia, 3 (213) (2007) 339.
6. Krzeminski K., Rudniak L. and Dahlke M., Tribologia, 1 (193) 2004.
7. Trzaskacz T., Tribologia, 4 (193) 2004.
8. Adamson A.W., Physical Chemistry of Surfaces. Wiley and Sons, 1976.
9. Burke S.E. and Barrett C.J., Biomacromolecules, 4 (2003) 1773.
10. Schlenoff J.B., Langmouir, 25 (2009) 14007.
11. Kato N., Schuetz P., Fery A. and Caruso F., Macromolecules, 35 (2000) 9780.
12. Petelska A.D. and Figaszewski Z.A., Biolectrochem. Bioenerg., 46 (1998) 199.
13. Pawlak Z., Kotynska J., Figaszewski Z.A., Gadomski A., Gudaniec A. and Oloyede A., Archives Mat. Sci. Eng., 29 (2008) 24.
14. Petelska A.D., Naumowicz M. and Figaszewski Z.A., In: Advances in Planar Lipid and Liposomes, eds. H.T. Tien and A. Ottova, Elsevier; vol. 3 (2006) p.125–187.
15. Hills B.A. and Crawford R.W., J. Arthroplasty, 18 (2003) 499.
16. Pawlak Z. and Oloyede A., BioSystems, 94 (2008) 193.
17. Singiel D.P., Biophys. J., 45 (1984) 399.
18. Gadomski A., Pawlak Z. and Oloyede A., Tribol. Letters, 30 (2008) 83.
19. Higaki H., Murakami T., Nakanishi Y., Miura H., Mawatari T. and Iwamoyo Y., Proc. Inst. Mech. Eng., 212 (1998) 23.
20. *Trunfio-Sfarghiu A.M.*, Berthier Y., Meurisse M.H. and Rieu J.P., Langmuir, 24 (2008) 8765.
21. Pawlak Z., Crawford R.W. and Oloyede A., Australian J. Mech. Eng., 6 (2008) 21.
22. Jurvelin J.S., Müller D.J., Wong M., Studer D., Engel A. and Hunziker E.B., Surface and subsurface morphology of bovine humeral articular cartilage as assessed by atomic force and transmission electron microscopy, J. Structural Biology, 117 (1996) 45.
23. Jurvelin J.S., Prediction of biomechanical properties of articular cartilage with quantitative magnetic resonance imaging, J. Biomechanics, 37 (2004) 321.
24. Pawlak Z., Tribochemistry of Lubricating Oils, Elsevier, 2003.
25. Gale L.R., Coller R., Hargreaves D.J., Hills B.A. and Crawford R., Tribol. Intern., 40 (2007) 601.
26. Furey M.J. and Burhardt B.M., Lubrication Sci., 9 (1997) 255.

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Streszczenie

Mechanizm smarowania i tarcia w stawach naturalnych opisany jest w literaturze przez ok. 40 modeli. Znaczny postęp w ostatnich latach zawdzięczamy badaniom eksperymentalnym prowadzonym na poziomie molekularnym. Staw, nazywany biołożyskiem, cechuje ultra-niski współczynnik tarcia rzędu 0,001, często pod obciążeniem do 20 MPa. Maź stawowa (łac. synovia, płyn maziowy) jest środkiem smarującym (pH ~ 7,4) zawierającym ok. 94% wody, chondrocyty, fosfolipidy o m.cz. 700, biomakrocząsteczki, m.in. lubrycyne, hialuron o m. cz. ok. 200 000. W pracy przedstawiono wpływ pH na hydrofobowość, napięcie powierzchniowe, gęstość ładunku i współczynnik tarcia powierzchni stawu. W naszym micelarno-lamelarnym modelu smarowania dwuwarstwy (membrany) fosfolipidowe (SAL-surface amorphous layer) osadzone są na chrząstce stawowej i z udzialem biomakrocząsteczek zapewniają ultraniskie tarcie. Przy spadku hydrofobowości chrząstki z 105° do ok. 60° i zakłóceniu równowagi kwasowo-zasadowej SAL się nie odtwarzania i tarcie w stawie wzrasta.