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Journal of Clinical Case Reports and Studies
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Research Article

The amphoteric cartilage surface tested in the pH Range from 2.0 to 9.0

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Received date: September 20, 2021: Accepted date: October 09 2021: Published date: January 03, 2021

Citation: Michal Sojka and Zenon Pawlak, (2022) The amphoteric cartilage surface tested in the pH Range from 2.0 to 9.0. *J. Clin Case Rep and Stu* 3(1); DOI: 10.31579/2690-8808/092

Abstract.

Background: Phospholipids adsorbed to negatively-charged proteoglycan matrix form phospholipid (membrane), have negatively charged surface ($-PO_4^-$) and are hydrophilic. Strong adsorption and strong cohesion are necessary for phospholipids to provide a good lubricant. The surface energy of spherical lipid bilayers have "bell-curve" shaped has amphoteric character and lowest surface energy at a pH 7.4 ± 1 of the natural joint.

Objectives: The amphoteric character of the natural surface of the articular cartilage was determined by measuring the surface energy of the model spherical bilayer lipid membrane. It was found that the friction *(f)* vs. pH 2.0 to 9.0 of the pair (cartilage/cartilage) has the amphoteric character by exposing "bell-curve" shaped with an isoelectric point (IEP).

Methods: The friction coefficient *(f)* was measured with the sliding pin-on-disc tribotester the friction between two surfaces (cartilage/cartilage) pair. The method of interfacial tension measurements of the spherical lipid bilayer model vs the pH over the range 0.2 to 9.0 was used.

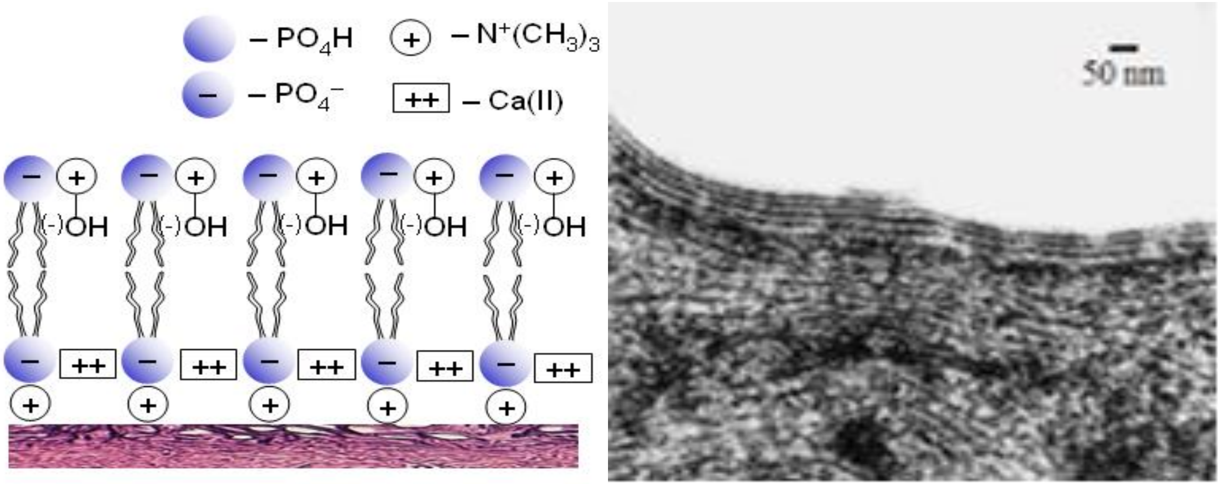
Results: The dependence of friction coefficient between two cartilage surfaces on the pH over the range 2.0 to 9.0 is demonstrated by a “bell - curve” in Fig. 2(A). The surface energy of a model spherical bilayer lipid membrane vs. the pH has the character of a “bell - curve” with an (IEP) is shown in Fig. 2(B).

Conclusion: The amphoteric effect on friction between the bovine cartilage/cartilage contacts has been found to be highly sensitive to the pH of an aqueous solution. In this paper we demonstrate experimentally that the pH sensitivity of cartilage to friction provides a novel concept in joint lubrication on charged surfaces. The change in friction was consistently related to the change of charge density of an amphoteric surface.

Keywords: amphoteric cartilage; phospholipid bilayers; friction coefficient; surface energy; pH

Introduction

Phospholipids are amphoteric molecules containing both positive and negative charges depending on the functional groups, which is affected by the solution’s pH [**1**]. Liposomes and phospholipid lamellar phases are structures composed in the synovial fluid and the bilayers adsorbed on the surface of the cartilage play the role of a lubricant [**1-4**]. Types of phospholipid adsorbed onto the surface of cartilage were identified: phosphatidylcholine (41%), phosphatidylethanolamine (27%) and sphingomyelin (32%) which were the major components of the lipid bilayers on the normal cartilage surface [**5**]. Phospholipids adsorbed to negatively-charged proteoglycan matrix form phospholipid (membrane), have negatively charged surface ($-PO_4^-$) and are hydrophilic. Strong adsorption and strong cohesion are necessary for phospholipids to provide a good lubricant. Molecular model for adsorption of a cationic quaternary ammonium (QA) ion to the hydrophilic surface and cohesion barrier imparted by phosphate to phosphate bonding by Ca (II) cation is shown in Fig. 1(A). The surface energy of spherical lipid bilayers evident from phosphatidylcholine "bell-shaped curve" has amphoteric character and lowest surface energy at a pH 7.4 ± 1 of the natural joint.



(A) (B)

Fig. 1 *(A) Hydrophilic cartilage model with the negatively charged surface for pH 7.4 with strong adsorption (-N+(CH3)3 – (proteoglycan) and strong cohesion (-PO4-) (Ca2+)(-PO4-) (B) An image made by electron microscopy of human knee cartilage with phospholipid bilayers adsorbed on the surface [3].*

It was shown that the pairs (cartilage/cartilage) charged positively or negatively with a comparable charge density had an equivalent value of the coefficient of friction. The surface energy of a model spherical bilayer lipid membrane vs. the pH has the character of a "bell curve". A significant dependence of friction on the cartilage charge density and wettability of the surface is to be expected. The natural cartilage of the animal joint has very low friction for low sliding speed – a few centimeters per second under a load of 18 MPa [**6**].

In this work the amphoteric character of the natural surface of the articular cartilage was determined by measuring the surface energy (denoted by (γ)) of the model spherical bilayer lipid membrane and by measuring the friction *(f)* of the pairs (cartilage/cartilage) positively (+ / +) or negatively (- / -) charged and uncharged the surfaces (±) / (±) for IEP in aqueous buffer solution for pH 2 to 9. It was found that the friction of the pairs (cartilage/cartilage) depends on the charge density of the cartilage surface and moreover, that charged surfaces have the lower coefficient of friction than the neutral pair (cartilage / cartilage).

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. Then, the articular cartilage discs were cut in such a way to form 3-mm plugs with underlying bone. Then, the samples were stored at 253 K in 0.15 M NaCl (pH = 6.9) solution and before testing they were fully defrosted. Next, the discs of articular cartilage were glued to the disc and attached to the surface made of stainless steel. Finally, friction tests were carried out.

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 acid. A sodium hydroxide solution was used at the temperature 22^{o}C to adjust a suitable buffer pH [7]. The electrolyte pH was controlled using a pH-meter in the process of the measurements.

The method of measuring the surface energy of the spherical lipid bilayer model: Experimental conditions of the surface energy measurement of the spherical lipid bilayer model vs. the pH are described in detail in the papers [**8, 9**].

The determined physicochemical parameters (isoelectric point, (IEP) and surface energy (γ) mJ/m^2) have values: phosphatidylcholine (IEP = 4.12, (γ) = 3.53), phosphatidylethanolamine (4.18, 4.06), phosphatidylserine (3.80, 2.93) and sphingomyelin (4.01, 4.42) (cited from Table 1[**9**]. Using literature experimental data curve Fig. 2B was obtained. The measurements of interfacial energy as a function of pH are shown in Fig. 2B.

Friction test

The friction coefficient *(f)* was measured with the usage of the ITeR sliding pin-on-disc tribotester at room temperature. The tribotester measured the friction between two surfaces (cartilage/cartilage) pair which were soaked in buffer solution the playing a role of lubricating fluid , and exposed to a load with sliding velocities for some time. The speed of sliding discs was very low (1mm/s in 10 min) and the load was 15 N (1.2 MPa), what is relevant to the natural physiological conditions. Before running the test, the specimens of articular cartilage were put to buffer solution for 10 min. Then, 5 tests were carried out with the usage of fresh articular cartilage discs for each experiment which is described in [**6**]

Results and discussion

The dependence of friction coefficient between two cartilage surfaces on the pH over the range 2 to 9 is demonstrated in **Fig. 2(A). Fig. 2(B)** shows the "bell curve" of the surface energy of the spherical bilayer as a function of pH. The isoelectric point of four phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin) is approximately equal 4 ± 0.2.

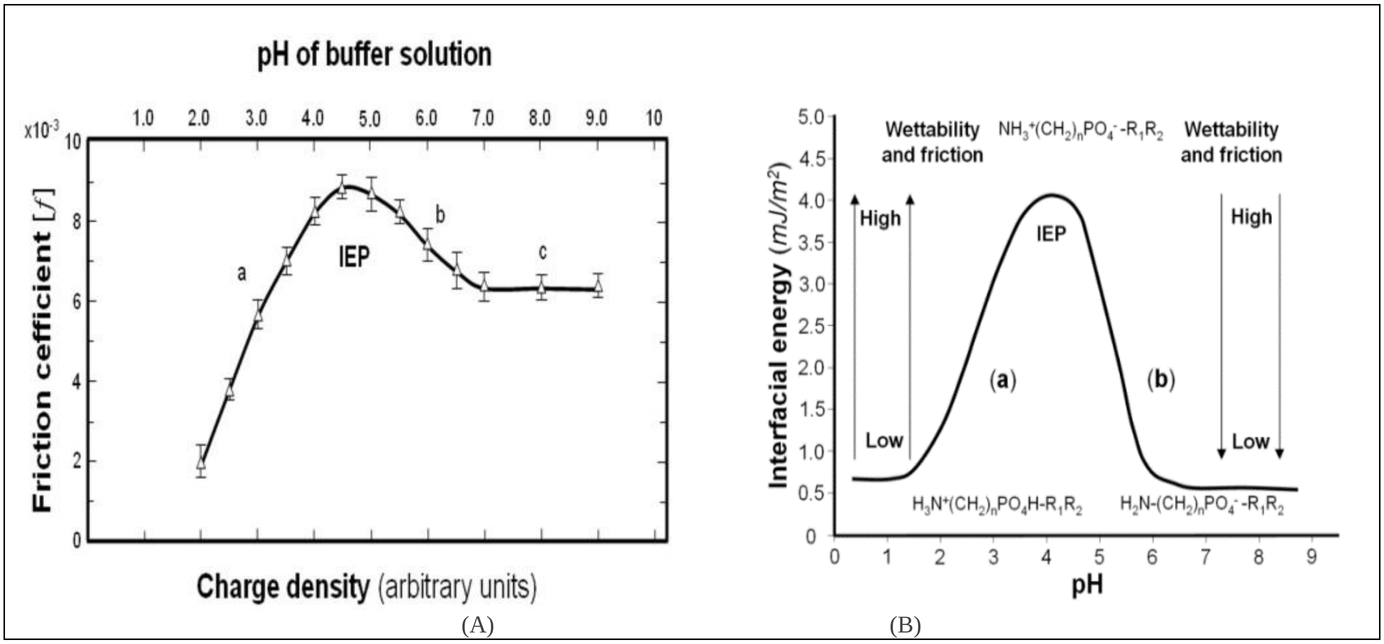


Fig. 2. *(A) Coefficient of friction on the surface of tribological pair (cartilage/cartilage) vs. the pH of the buffer solution. (B) The surface energy of a spherical phospholipid bilayer presented in the form of a „bell curve". Spherical bilayers formed from phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin) tested in a buffer solutions with a pH range of 0.2 to 9.0. Curves (a) and (b) describe the reaction of functional groups:*

(a) ($-NH_3^+$) → $-NH_2$) as pH grows **(b)** ($-PO_4H$ → ($-PO_4^-$) as pH grows.

In Fig. 2(A) the friction results in low acidic pH below 3 are lower as compared to higher 5 to 7 pH and this is related to cartilage swelling and giving "bell curve" asymmetric. 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 [**10**] and above this value, it was constant. Under most conditions, softening was associated with increased hydration with a reduction in friction.

In **Fig. 2 (A)** the coefficient of friction and (**B**) the surface energy curves have an isoelectric point (IEP) (the maximum on the curves A and B), which is a state of the electrostatic equilibrium at which the phospholipid molecule does not carry any net electric charge. The bell curve describes the amphoteric nature of the phospholipid membrane; e.g. Phosphatidylethanolamine.

IEP : $H_2N\,(CH_2)_n\,PO_4H\text{-}R_1R_2 \rightarrow H_3N^+(CH_2)_n\,PO_4^-\text{-}R_1R_2$

(a) (pH 0.2 to 4.0) $H_3N^+(CH_2)_n\,PO_4H\text{-}R_1R_2$; (a) (Left side of the curve)

(b) *(pH 4.0 to 6.5)* $H_2N\,(CH_2)_n\,PO_4^-\text{-}R_1R_2$ (b) (Right side of the curve)

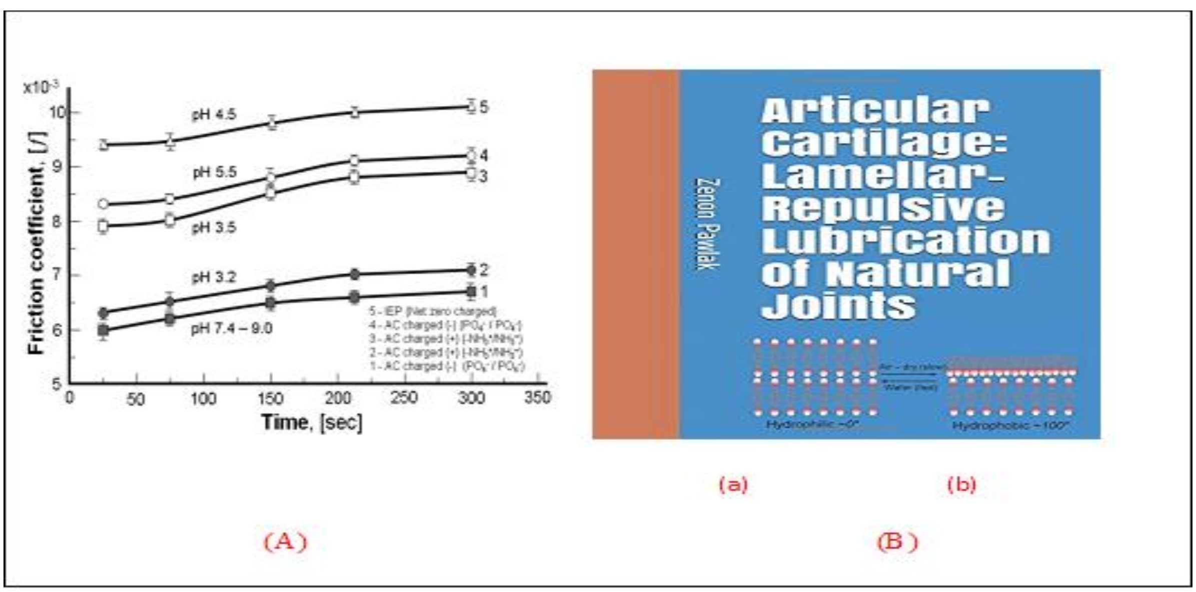


Fig.3.(B) *Friction coefficient (f)for the (cartilage /cartilage) tribopairs vs. time over the pH range 3.2 to 9.0 in the Britton-Robinson buffer solution under a 15N load and 1 mm/s sliding velocity during 300 seconds.*

Fig. 3 (A) Friction coefficient f for the (cartilage /cartilage) tribopairs vs. time over the pH range 3.2 to 9.0 in the Britton-Robinson buffer solution under a 15N load and 1 mm/s sliding velocity during 300 seconds. (B) Variations in surface energy lead to conformational transformations in the surface phospholipids from bilayer (hydrophilic) to monolayer (hydrophobic). Phospholipid bilayers of articular cartilage under the wet (a) and air-dry (b) conditions and the lipid flip-flop is affected by dehydration activated process.

In Fifure 3(A) Before IEP (Curves 2 and 3 run at pH 3.2 and 3.5) each pair charged positively (+) on ($-NH_3^+/NH_3^+$). After IEP (Curve 1 and 4 run at pH7.4 and 5.5 each pair charged negatively (-) on ($-PO_4^-$ / $-PO_4^-$) At IEP (curve 5) at pH 4.5 both surfaces at net zero charges. The standard deviation (SD) of the friction coefficient *f* ranges from 10 to 15 %.

Negatively charged surfaces (cartilage/cartilage) with synovial fluid pH 7.4 under load are involved in the lamellar slip of bilayers with low friction. It has been demonstrated experimentally that lamellar phospholipid phases in the synovial fluid with bilayers on the AC surface play a vital role as a lubricant. The amphoteric surface of cartilage tested in the pH range from 2 to 4.2 disposes of a positive charge ($-NH_3^+ \rightarrow -NH_2$), and an increase in buffer pH charges the cartilage surfaces negatively ($-PO_4H \rightarrow -PO_4^-$-). The coefficient of friction of the pair (cartilage/cartilage) was measured for three cases: both cartilages were positively charged ($-NH_3^+$)/($-NH_3^+$); negatively charged ($-PO_4^-$) / ($- PO_4^-$) and in the neutral state of both surfaces (isoelectric state, IEP). It was shown that the charge of the cartilage surface is an essential parameter in the friction process. Friction study (cartilage/cartilage) of bovine cartilage parties with the use of buffer solutions allows observation of the mechanism of electrostatic lubrication of joints [**11,12**]

Hydrophilic and negatively charged cartilage surface ($-PO_4^-$) for pH 7.4 is well hydrated and lubricated via lamellar-repulsive mechanism. Synovial fluid macromolecules lubricin and hyaluronan molecules also involved in adsorption with phospholipid can be attached to cartilage surface but the main role is phospholipid transportation [3].

The amphoteric effect on friction between the bovine cartilage/cartilage contacts has been found to be highly sensitive to the pH of an aqueous solution. In this paper we demonstrate experimentally that the pH sensitivity of cartilage to friction provides a novel concept in joint lubrication on charged surfaces. The change in friction was consistently related to the change of charge density of an amphoteric surface.

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DOI: 10.31579/2690-8808/092