

## Lipid Translocation (Flip-Flop) As One of the Least Understood Dynamical Processes in Articular Cartilage Membrane

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### Abstract

The lipids molecules translocation in phospholipid membrane is named the (flip-flop) event. For this purpose, the translocation may take place in vivo with the surface of articular cartilage. Flip-flops are one of the least understood dynamical processes in vivo in cartilage membranes. In this work, we explain the molecular mechanism lipid translocation (flip-flop) in vivo during the drying process cartilage surface. Wettability of cartilage surface vs. drying time was determined. Our findings strongly support the idea that the process of translocation lipid (flip-flop) transformed hydrophilic surface in hydrophobic in dry-air condition for healthy and osteoarthritic cartilage. Such material can be classified cartilage as “smart material” and this fact is poorly known in the literature.

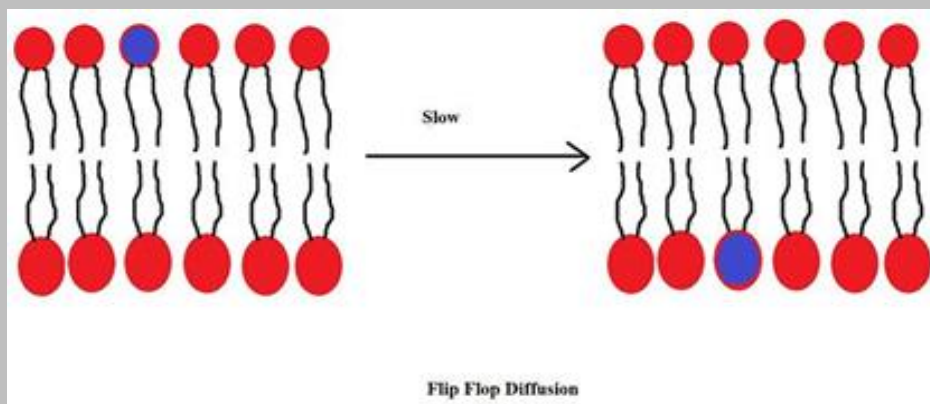
**Keywords:** Transformation flip-flop; Cartilage; Wettability; Hydrophilic; Hydrophobic

### Introduction

Phospholipids molecules in aqueous media spontaneously arrange into a bilayer on the surface of articular cartilage. The bilayer is held together by hydrophobic interactions between the tails. Phospholipid (PL) bilayers on the surface of articular cartilage provide characteristics that are well adapted to wet and relatively dry conditions. This smart surface characteristic creates a hydrophobic-hydrophilic balance resulting in a functional hydrophilic surface in the intact joint. One of the quantitative indicators of surface tribochemical properties is wettability. This is measured as the contact angle between a drop of water and the reference surface.

Wettability characterizes the surface of various objects, which are generally defined as wettable (highly hydrophilic,  $\theta \sim 0^\circ$  to  $45^\circ$ ) or non-wettable (highly hydrophobic,  $\theta \sim 90^\circ$  to  $180^\circ$ ).

The mechanisms to actively transport lipids across a lipid bilayer using specific membrane are widely recognized [1]. All lipids in biomembranes can move within the bilayer, allowing for membrane fluidity and flexibility. Exchanging places with their neighbors by slow transverse diffusion (see Fig. 1) and fast lateral shift ( $\sim 10^{-6}$  sec). Double bonds in unsaturated hydrocarbon chains also tend to increase fluidity of the phospholipid bilayers. This fluidity allows for the spontaneous breaking and reforming of membranes [1, 2]. This is called flip-flop mechanism. Membrane fluidity is enhanced at higher temperatures and is also affected by the composition of the bilayer. The formation of a transient water pore (defect) in the membrane inevitably leads to diffusive translocation of lipids through the pore, which is driven by thermal fluctuations (Fig.1) [3].



**Fig.1.** The motion of phospholipid with lipid bilayer in aqueous solution. Transverse diffusion (flip-flop) is slow ( $\sim 10^5$  sec).

The experimentally observed fact that the exposure of lipid membranes to electric field pulses considerably reduces the time required for lipid flip-flops. Negatively charged surface of articular cartilage membrane inevitably leads to diffusive translocation of lipid flip-flop.

In this work, wettability of cartilage surface vs. drying time was determined. Wettability helps us to explain the molecular mechanism lipid translocation (flip-flop) for healthy and osteoarthritic cartilage. Lipid translocation in cartilage membranes is still far from being understood and characterized. It is observed that lipid flip-flop is affected by dehydration activated process. Moreover, we report for the first time wettability of unhealthy cartilage in vivo changes own characteristics.

## Experimental

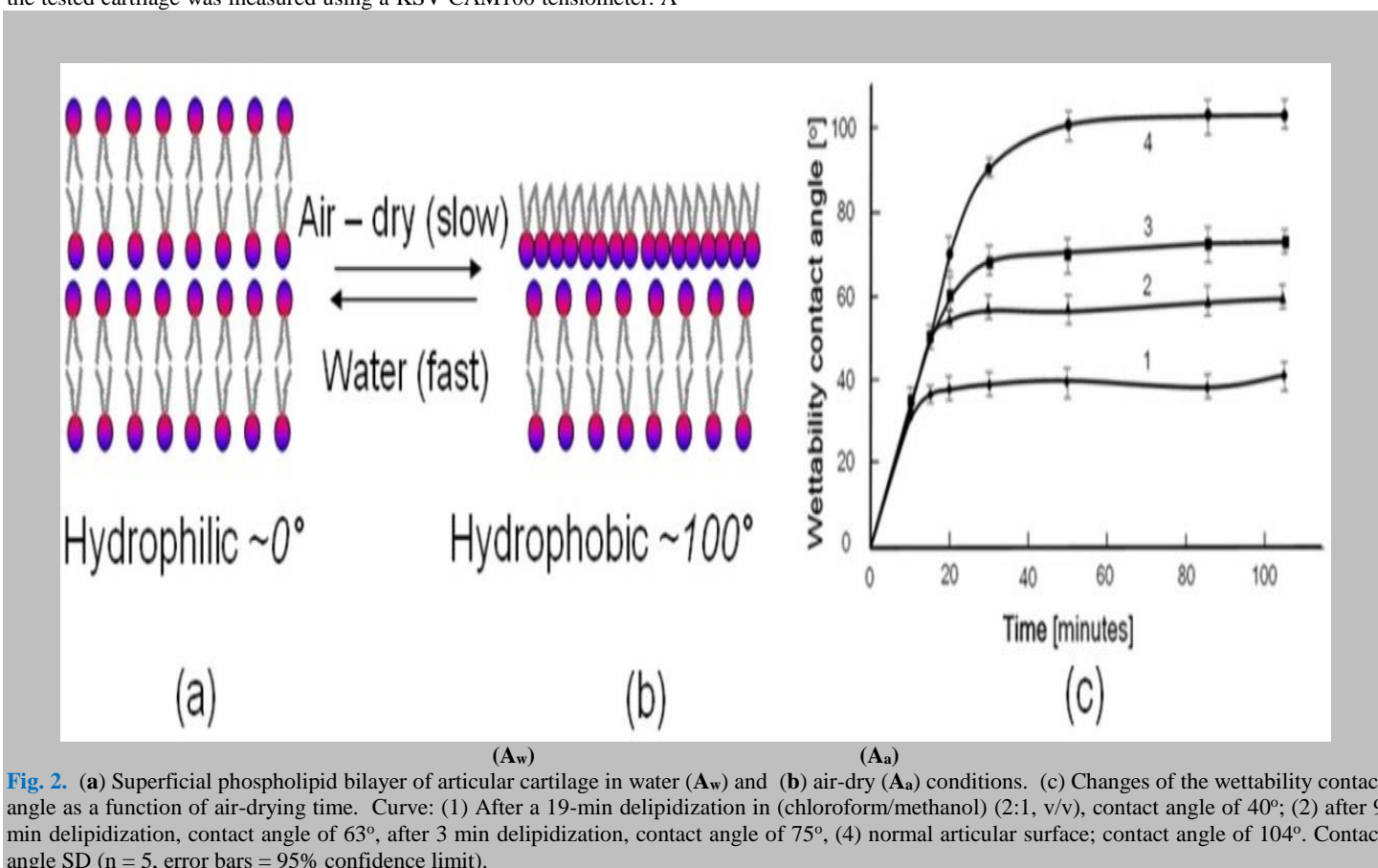
In the delipidization procedure a Folch reagent (2:1 v/v mixture of chloroform and methanol) was used to gradually remove the PL bilayers from the bovine cartilage surface. The samples were immersed in the reagent mixture for 3, 9 and 19 minutes at the same meniscus. After extraction, the sample was placed in saline solution for 1 hour to remove the residual solvent and promote rehydration and then air-dried. These samples were used for the surface wettability and friction measurements. The delipidization procedure removed most of the PL, although some amount of a hydrophobic proteolipid remained as a minor component [4].

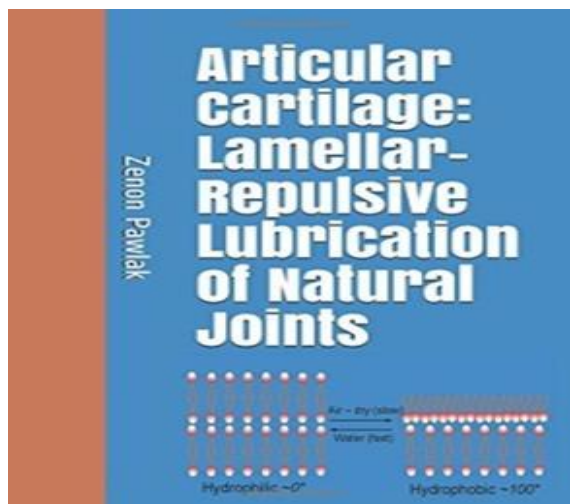
**Contact angle measurements** The contact angle between the liquid and the tested cartilage was measured using a KSV CAM100 tensiometer. A

tensiometer is a device for measuring the surface tension of a liquid. The contact angle measured was that between a droplet of a 0.15M saline solution and a given air-dry cartilage surface. The contact angle measurements of the normal (intact) and the delipidated cartilage samples were carried out under dry-air atmosphere in the laboratory [5, 6] at ambient temperature of 22°C and a relative humidity (HR) of approximately 45 %. Five tests were performed on each specimen and each set-up. The contact angle test performed on the normal, partial and completely depleted cartilage samples. The depleted cartilage samples imitate osteoarthritic degradation of cartilage surface.

## Results and discussion

Formation of structural defects (water pore) is the result of an increase in the lateral surface tension induced by the evaporation surface water. This surface tension produced by formation of a transient water dehydration in the membrane inevitably leads to diffusive translocation of lipids (flip-flop) can in turn induce changes from bilayer to monolayer (**Fig. 2**). A change in surface energy leads to conformational changes in the surface of bovine patella from bilayer (super hydrophilic  $\sim 0^\circ$  contact angle) to monolayer (hydrophobic  $\sim 104^\circ$ ). Changes of the wettability contact angle as a function of air-drying time are shown in Fig.2 (c) with totally depleted surface of phospholipid bilayer curve (1) contact angle of  $40^\circ$ ; partially depleted PL curve (2) and (3) contact angle of  $63^\circ$ , and  $75^\circ$ , and curve (4) normal articular surface; contact angle of  $104^\circ$ .





**Fig. 3.** Phospholipid bilayers of articular cartilage under the wet and air-dry conditions. Variations in surface energy lead to conformational transformations in the surface phospholipids from bilayer (hydrophilic) to monolayer (hydrophobic). Book cover “Articular cartilage: Lamellar-repulsive lubrication of natural joints” [6].

The wetting studies on healthy cartilage and osteoarthritic surface *in vivo* indicate that, when exposed to dry atmospheric conditions, only hydrophobic chain is exposed to the outside. When the surfaces are becoming exposed to bulk water the surfaces rapidly become hydrophilic (Fig. 2). These findings are entirely consistent with those of the adhesion measurements [7, 8].

### Conclusions

We studied how the wetting properties of the cartilage surface *in vivo* changed with dehydration. The results are described and summarized in

Fig. 2. It has been demonstrated that the “smart surface” of cartilage is super hydrophilic when wet and hydrophobic when air-dry, **Fig.3**. The key to understanding the mechanism of joint lubrication lies in obtaining an insight into the relationships between the structure and function of surface. The main result of the present study is the observation that the rate of lipid flip-flops is significantly enhanced by the spontaneous formation of water pores during dehydration. Evidently, one should then ask whether the rate of pore formation is comparable to the lipid flip-flop rate in the absence of pores. It is observed that lipid flip-flop is affected by dehydration activated processes.

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