Experimental Report

Proposal: **8-02-673** **Council:** 10/2012

Title: Vertical density profile of two phospholipid bilayers in a model contact of articular joint as a function of the applied pressure

This proposal is a new proposal

Researh Area: Biology

Main proposer: **RIEU Jean-Paul**

Experimental Team: SFARGHIU-TRUNFIO Ana-Maria
FRAGNETO Giovanna
RIEU Jean-Paul
DER LOUGHIAN Christelle
MURALI MOHAN Meera
PETIT Alexandre

Local Contact: GUTFREUND Philipp

Samples: DLPC, DPPC, PAA hydrogel

Instrument	Req. Days	All. Days	From	To
FIGARO	3	3	23/07/2013	26/07/2013

Abstract:

The purpose of this proposal is to measure the full vertical density profile of a stack of two bilayers in contact immersed in various water solutions, one supported on a silicon substrate, and the other on a soft compliant layer of hydrogel as a function of these parameters.

Experimental Report on Experiment Number: 8-02-673

"Vertical density profile of two phospholipid bilayers in a model contact of articular joint as a function of the applied pressure"

Participants: J.-P. RIEU (1), M. MURALI-MOHAN (1), Alexandre PETIT (1), G. FRAGNETTO (2)

(1) Institut Lumière Matière, Lyon, France; (2) ILL, France

Local contact(s): GUTFREUND Philipp, gutfreund@ill.eu

Instrument FIGARO from 23/07/2013 8:30am to 26/07/2013 8:30am

Context

Nature has produced water-based lubricant systems that far outclass the best of most man-made devices [1]. Biological contacts such as the articular cartilage surfaces in human hips or knees often operate under severe conditions (*i.e.* high load and low speed), which is related to a boundary lubrication regime characterized by a very low friction coefficient (μ=0.005–0.02, for the human joint). Breakdown of this lubrication can lead to wear of the cartilage and to osteoarthritis. Insights into the molecular origins of cartilage lubrication could lead to more efficient medical treatments, tissue repair and to longer-lasting prostheses.

Studies have sought to identify the component of synovial fluid capable of playing the role of boundary lubricant. It was shown that the samples of synovial fluid contain phospholipid molecules (mostly choline) [2]. A stacking of 3 to 7 phospholipid bilayers separated by aqueous layers has been highlighted in almost all biological in-vivo rubbing surfaces and proposed to reduce the friction between biosurfaces [3]. Experiments carried out in Lyon (a collaboration between ILM/UCBL and LaMCoS/INSA de lyon) have confirmed this assumption using a home-made bio-tribometer constituted of two model hydrophilic surfaces (glass and soft hydrogel of pHEMA) covered each by a phospholipid bilayer [4-6]. The friction coefficient of model surfaces is reduced by nearly 2 orders of magnitude, to a very reproducible value μ=0.002 when surfaces are covered by a DPPC bilayer (gel phase). With only one bilayer in the contact region (gel or fluid phase), the friction coefficient was much higher and bilayers quickly degrade indicating that lubrication is ensured by the hydration layers between adjacent lipid bilayers [4]. Fluid bilayers are intrinsically less lubricant than solid ones suggesting that either lipid mobility or protrusion modes impact lubrication [6].

In order to interpret these experimental results and try to model them, it is necessary to know the vertical density structure within the contact region (bilayer thickness and roughness, water interbilayer or substrate-bilayer thicknesses), its dependence on normal load, shear velocity and physico-chemical parameters (ions, temperature). The purpose of this proposal was hence to measure the full vertical density profile of a stack of two bilayers in contact immersed in various water solutions, one supported on a silicon substrate, and the other on a soft compliant layer of hydrogel as a function of these parameters.

Methods

We recorded the specular reflectivity in the contact between two supported bilayer pressed against each other thanks to the Anton-Paar rheometer setup of Figaro line: the first bilayer was deposited directly on a highly polished crystalline silicon (111) substrate (rms roughness of 1.5Å) glued on the bottom rheometer plane; the second bilayer was deposited on thick polyacrylamide (PAA) or pHEMA hydrogel layers prepared in Lyon before the experiment and mounted on the upper rheometer plane. The incubation was done *in situ* in the rheometer according the following protocol (figure.1). The lipid vesicles solutions used were POPC and DPPC with a 0.5 mg/ml concentration and containing vesicles measuring 100 nm in diameter.

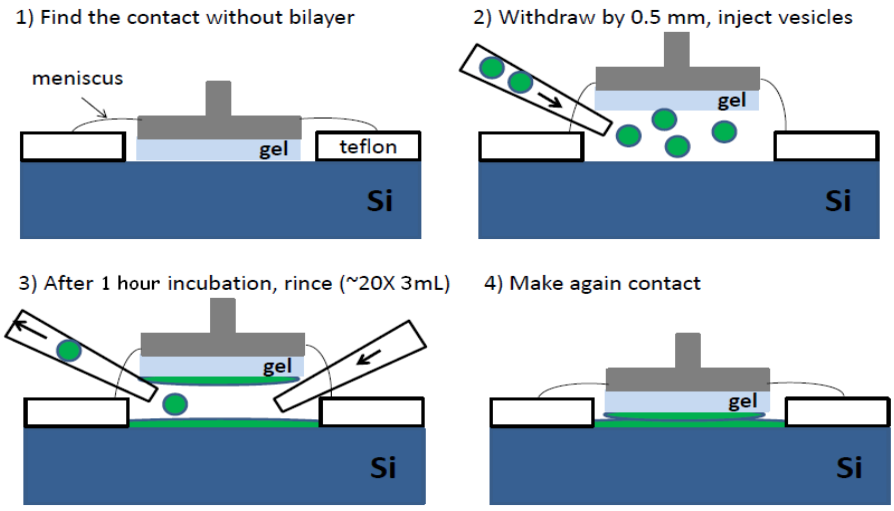

Figure.1: *Experimental protocol used for the rheological experiments under the rheometer. After finding the gap without and with the gel (1), the two surfaces were separated by 0.5 to 1 mm and 4-6 ml of vesicles solution were injected(2). The incubation lasted 1hour and the solution was rinsed with about 50ml of solvent (D_20 or H_20) (3) before the surfaces were contacted again for the experiment (4).*

We also prepared DMPC multi-bilayers (~1000 bilayers) by solvent extraction. For this purpose, 1.2 ml of DMPC at 20 mg/ml was diluted into isopropanol (100%). The mixture was poured on the surface of the silicon crystalline block and then dried under vacuum for 2-3 hours. The wet gel was approached and contacted with the Si supported dried surface and finally solvent was added.

We investigated the following range of normal forces: 1 and 50 N of force. Even if the contact geometry (plane-plane) was not perfectly controlled, we could estimate the contact pressure using the gel diameter of about 5 cm before compression.

Results

We have validated during that run of experiments the method of preparation and the sample cell (with a Teflon ring and a solvent trap not represented in Figure 1) to keep hydrated during a long time the two contacting surfaces. We were initially a bit disappointed by the results as we could not for any pressure observe the very characteristic vertical density profile of two stacked bilayers [7]. We mostly measured only one bilayer, the one on the Si substrate whatever the hydrogel type and the lipid type (DPPC in the gel phase or POPC in the fluid phase). However, at two occasions, we started to see at large normal forces a Bragg peak at Q_Z~0.1 $Å^{-1}$ characteristic of a multibilayer stacking. We checked that point by depositing a multibilayer and sandwiching with the gel surface and obtained the same kind of signal. We tried to study the response under shear of this multilayer but the gel did not resisted sufficiently to get convincing results.

Conclusion and perspectives

It seems that neutron reflectivity is really the tool of choice to measure the vertical density of two or several sandwiched bilayers. However, as these experiments are very time consuming, we are currently in Lyon improving hydrogel fabrication (both its mechanical strength and its roughness) and trying to estimate the distribution of contact area using FRET and RICM optical microscopy techniques. We are also staring to work on sandwiched multibilayers that seem a promising system, and in the near future, we would like certainly to perform another neutron experiment.

References

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