Experimental Report

Proposal: **8-02-646** **Council:** 4/2012

Title: Mechanical properties of bio-membranes interacting with a model synovial fluid

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples: Silicon single crystal (solid support)
D2O
Hyaluronic acid: (C14H21NO11)n
1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC)

Instrument	Req. Days	All. Days	From	To
D16	5	5	24/10/2012	29/10/2012

Abstract:

Ultrathin lipid linings are terminal layers on articular cartilage and are of outmost importance for lubrication in mammalian joints. We will apply specular and offspecular neutron scattering to solid-supported, hyaluronic acid (HA)-interlinked DMPC oligo-bilayers against excess aqueous liquid phases (HA+D2O). The experiments will yield valuable insight into the microscopic mechanical behavior of the lipid coatings and thereby into the underlying mechanism responsible for lubrication. We will extract the compression- and bending moduli of the HA-interlinked lipid membranes by adapted modelling of the shape of the recorded Bragg-sheets in two dimensions (qz and q||). With a preset but varied number of lipid bilayers in the stacks we will focus on the influence of HA and temperature on the system mechanics.

Proposal Number 8-02-646

Mechanical properties of bio-membranes interacting with a model synovial fluid

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Introduction

One of the main components of synovial fluid is hyaluronic acid (HA), a high molecular weight polyelectrolyte [1]. HA is involved in a wide range of processes in the human body, such as wound healing, tumor progression and joint lubrication [2-4]. As for the latter, also phospholipids play an important role. In mammalian joints they cover the cartilage of opposing bones and are also part of the synovial fluid that fills the joint cavity [5]. It is claimed that in particular the combination of phospholipids and HA into composite layer structures plays an import role in the lubrication properties of the joint [6]. Investigations on animal joints confirmed that intra-articular injections of phospholipids and HA reduce friction by acting as a boundary lubricant, thus protecting articular cartilage from degenerative changes in artificially induced experimental osteoarthritis [4]. A possible explanation of these findings may base on the micro-mechanics of such oligolayers. However, studies addressing the microscopic mechanical properties of the phospholipid cover layers in particular upon interaction with HA and other types of polyelectrolytes are still missing.

Results

In recent combined neutron reflectivity and infrared spectroscopy experiments we utilized solid-supported DMPC oligo-bilayers as model systems for lipid-coated cartilage/bone or lipid-coated artificial implants exposed to the fluid environment in mammalian joints [7]. The oligo-bilayers were immersed in pure water and high molecular weight HA solution, respectively. From the conducted experiments we achieved insight into structure, stability and interaction of the oligo-bilayers with the respective liquid environment on the molecular scale. When exposed to pure water at 21°C, i.e. below the main phase transition of DMPC, the coatings showed a lamellar spacing of 65 Å characteristic of the $L_\beta/P_{\beta'}$ state of the lipid system. Heating to 26°C resulted in unbinding and irreversible loss of the oligo-bilayers (except for one bilayer directly adsorbed to the substrate). On the contrary, when incubated in HA-solution and heated to physiologically relevant temperature above 37°C (39°C), the oligo-bilayers adapted a stable new state with a d-spacing of 247 Å.

In the experiment we report here, we investigated the mechanical properties of solid-supported, polymer-interlinked DMPC oligolamellar bilayers by off-specular neutron scattering [8-10]. In order to avoid the long equilibration times of the DMPC/HA system, a different polyelectrolyte was chosen for the experiment, namely poly(allylamine-hydrochloride) (PAH). This positively charged polymer proofed to reduce the equilibration time from weeks to days, by resulting in an equivalent swelling behavior of the lipid stack with a final d-spacing of 240 Å.

Sample Preparation

Oligolamellar DMPC lipid coatings (Sample I and III) were prepared by spin coating on pre-cleaned 615 µm thick silicon wafers. Furthermore, for comparison two multilamellar lipid coatings were prepared on similar silicon substrates by the air brush technique (Sample VII and IX). All samples

were pre-characterized by X-ray reflectometry in their dry state, right after sample preparation (data not shown). The reflectivity profiles of all samples show Bragg peaks up to the fourth order, resulting in a repeat distance of d = 52 Å. These results are in good agreement with literature for partially hydrated DMPC lipid membranes. In the following, samples I, III and VII were incubated one week before the neutron experiment in a solution of 3 mg/ml PAH in D_2O. Sample IX was incubated two weeks before the experiment in a solution of 3 mg/ml HA in D_2O.

Sample	Type	d- spacing in Å @ 20°C	d-spacing in Å @ 38°C
I	oligo vs PAH	200	202
III	oligo vs PAH	-	194
VII	multi vs PAH	143	143 (run 2)
IX	multi vs HA	-	60 (structure 1) 127 (structure 2)

Table 1

Results

A summary on the measured samples is shown in Table 1**.**

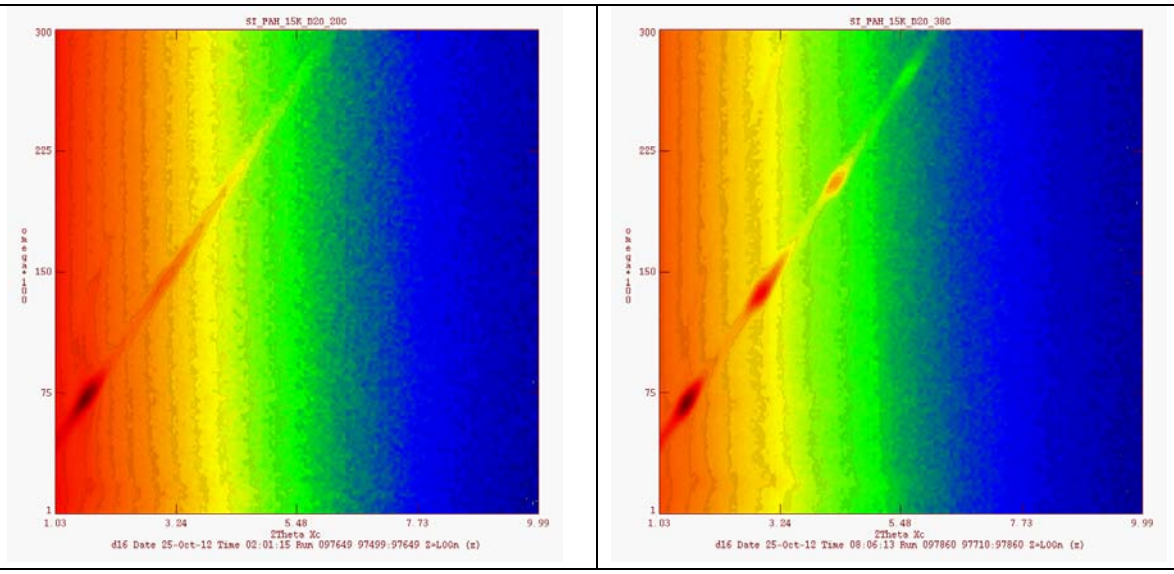

Figure 1: Neutron diffraction maps of Sample 1 against a solution of PAH in D_2O at 20°C (left) and 38°C (right).

The measurements of the samples against a liquid phase were performed at D16 at either 20°C or 38°C. The oligolamellar lipid coatings (Sample I and III) showed Bragg peaks up to fourth order (Figure 1). A preliminary Bragg peak analysis revealed a d-spacing of 200±10 Å at 20°C and at 38°C. The Bragg peak amplitudes are more intense at 38°C, but otherwise samples I and III show only weak off-specular scattering. In comparison, the multilamellar lipid coating, Sample VII, incubated in a solution of PAH showed Bragg peaks up to the second order (Figure 2), which revealed a d-spacing of 143±10 Å at 20°C and at 38°C. The Bragg peaks appear in combination with a strong off-specular signal. Sample IX, incubated in a solution of HA in D_2O, showed two Bragg peaks at 38°C (Figure 3), belonging to two different lamellar structures. The first peak reveals a d-spacing of 127±10 Å, resulting from a heavily swollen lipid coating. The second peak reveals a d-spacing of 60±10 Å, resulting from a fully hydrated DMPC lipid lining without polymer integration [7].

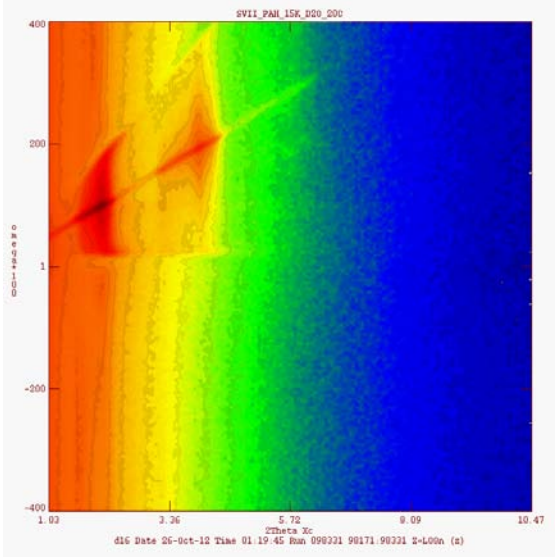

Figure 2: Neutron diffraction maps of the multilamellar lipid coating Sample VII against of a solution of PAH in D_2O at 38°C .

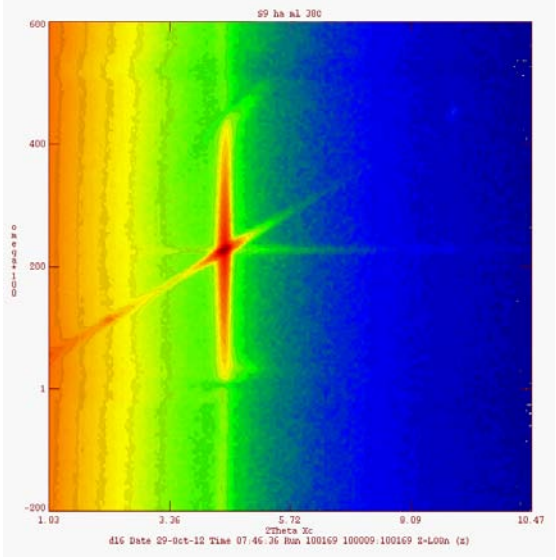

Figure 3: Neutron diffraction maps of the multilamellar lipid coating Sample IX against of a solution of HA in D_2O at 38°C.

Conclusion

Ultrathin lipid linings are terminal layers on articular cartilage and are of outmost importance for lubrication in mammalian joints. We applied specular and off-specular neutron scattering to solid-supported bilayers against excess aqueous solutions of polyelectrolytes (HA or PAH) in D_2O. The conducted experiments proof, that the presence of HA or PAH drastically increases the d-spacing of lipid coatings. As does the natural compound HA, also PAH stabilizes the lipid coatings above the main phase transition of the lipid molecules, in the liquid like phase of the oligolamellar lipid coatings at 38°C. Comparison between oligo- and multilamellar coatings shows, that while the oligolamellar coatings are already heavily swollen, the multilamellar coatings are still in a transition state after the same equilibration time. However, they have the advantage of showing highly pronounced Bragg sheets from which information on the microscopic mechanical parameters of the swollen lipid coatings will be extracted by line shape analysis. A respective analysis of the recorded off-specular data is currently under way. That analysis will yield information on the compression moduli and the membrane bending moduli of the investigated systems.

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