

Experimental report

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Proposal: 8-02-761

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Title: Neutron Diffraction Study of POPE Spray Coating under High Hydrostatic Pressure

Research area: Physics

This proposal is a new proposal

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Samples: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine

Instrument	Requested days	Allocated days	From	To
D16	5	5	16/06/2016	21/06/2016

Abstract:

Permanent metal implants are widely used and successfully implemented in medicine to address problems related to the musculoskeletal system. However, implants that interact in all cases optimally and durably with bone tissue have yet to be developed. To obtain biological active materials that provide biological cues for tissue regeneration, various coatings are applied. One of the best candidates for the implant modification is POPE lipid (phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine). The polar group of this lipid has been found in some nervous tissue, such as the white matter of the brain, nerves, neural tissue, and in the spinal cord. Besides, POPE coating has proved to be a positive factor for cell-implant interactions. However, it is extremely important to verify the resilience of the coating to mechanical damage. In order to fill this gap, the main goal of this project is to perform a structural study of POPE spray coating under high pressure, which mimics the mechanical stress in knees etc. The properties of POPE coating under high hydrostatic pressure is the key parameter for further applications on real implant materials.

Neutron Diffraction Study of POPE Spray Coating under High Hydrostatic Pressure

Over the last decades, permanent metal implants have become a successful tool in medicine to address problems related to the musculoskeletal system¹. However, there are still some important open questions, such as how to improve the performance of the implants and how to reduce the recovery time after a surgery. One of the most promising strategies is to apply a phospholipid coating on the metal surface to optimize the interaction with bone tissues. At this point, the resilience of the lipid coating under a mechanical stress at physiological temperatures higher than the phase transition is an important issue, since the coating should keep bone cells attached to a surface over weeks².

So far, lipid multilayer structures have been studied mostly as function of temperature, however, it is extremely important to verify as well the resilience of the coating against mechanical load³. In order to fill this gap, the main goal of this experiment was to perform a structural study of a lipid spray coating under high pressure, which mimics the mechanical stress in knees etc⁴.

Unfortunately, due to the lack of a sufficient quantity of POPE lipids necessary to obtain a reasonable signal, we had to switch to DMPC lipids to increase by 5 times the amount of the material in the high pressure cell, which was used for the first time for such an experiment. Therefore, a DMPC spray coating was prepared on two silicon wafers by an air brush connected to a nitrogen stream at pressure of 0.5 bar. 2 mL of the DMPC buffer solution containing a lipid concentration of 5 mmol DMPC per 1 L of solvent (20% methanol and 80% chloroform) was distributed over an area of 3 x 1 cm². After the lipid deposition, the two wafers were superposed to keep a sandwich like sample with DMPC lipids inside. In order to get the best diffraction signal from the lipid multilayer structure and to transmit homogeneously the pressure, an excess of pure D₂O was added to the sample. The newly developed high pressure cell with an aluminum insert including a rectangular slit permitted to investigate the membrane samples on a wafer without destroying it by the pressure application.

Omega scans from 0 to 16 degree showed the presence of Bragg diffractions (see Figure 1) up to the second order. Since higher Bragg diffractions were not distinguished, the rest of the data was collected in the omega angle range from 0 to 10 degree with a step of 0.1 degree (6 second counting time per step). To study the structural properties of DMPC spray coating under high hydrostatic pressure, we measured in the pressure range from 50 bar to 750 bar, which is the natural range of pressures reported for a knee⁴.

To determine the d-spacing of the DMPC bilayers, the collected scans were integrated over omega and then the maximum of the first order Bragg peak was determined by fitting it with a Lorentzian peak.

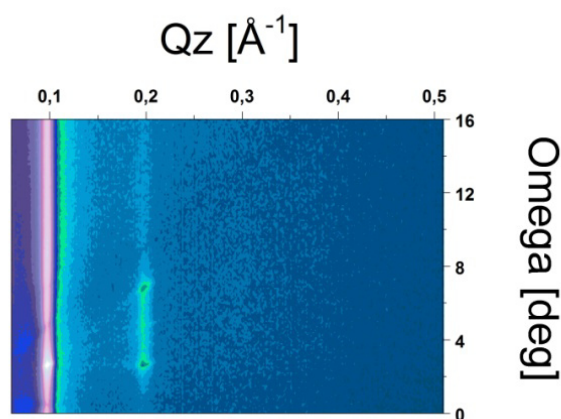


Figure 1. Omega scan of DMPC sample taken in the range from 0 to 16 degree at 292 K and 50 bar.

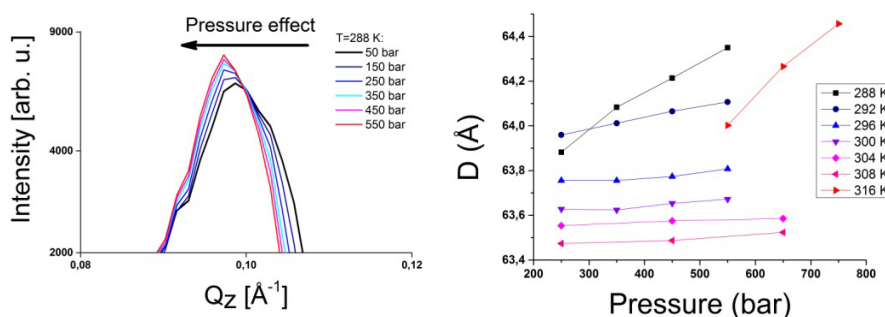


Figure 2. Pressure effect: left side - on the position of the first Bragg peak maximum at 288 K; right side – full pressure dependence of the d-spacing of a DMPC bilayer in the temperature range from 288 K to 316 K.

The data reveals that with the pressure increase the position of the first Bragg peak maximum shifts to smaller Q_z values indicating an increase of the DMPC bilayer thickness (d-spacing). At the same time, the pressure dependence of the d-spacing varies with the temperature change. For instance, the character of the pressure dependence of the d-spacing presents a clear modification at 296 K, which is the phase transition temperature of DMPC lipids. The second drastic change of the pressure dependence was observed at 316 K. We will have to investigate it further.

To conclude, here we report the DMPC multilayer structure and its properties under high hydrostatic pressure in the range of physiological temperatures, which is the key parameter for further applications on real implant materials.

References:

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3. Trunfio-Sfarghiu, A. M.; Berthier, Y.; Meurisse, M. H.; Rieu, J. P., *Langmuir* **2008**, 24 (16), 8765-71.
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