Experimental Report

14/10/2013

Proposal: **9-13-447** **Council:** 4/2012

Title: Variation of lipid layer thickness, internal structure and adhesion of proteins depending on the exposition to water and cell growth medium

This proposal is resubmission of: 9-13-403

Research Area: Soft condensed matter

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Samples:
$C_{2936}H_{4624}N_{786}O_{889}S_{41}$ Human Serum Albumin
$C_{2936}D_{4624}N_{786}O_{889}S_{41}$ deuterated Human Serum Albumin
$C_{39}H_{76}NO_8P$ -palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine
$C_{41}H_{12}NO_8PD_{70}$ distearoyl(d70)-sn-glycero-3-phosphoethanolamine
Si crystal

Instrument	Req. Days	All. Days	From	To
FIGARO	3	3	05/11/2012	08/11/2012

Abstract:

Permanent implants such as metallic hip prostheses are widely used and successfully implemented in medicine. The efforts are manifold to develop new implant surface modifications which may prolong the life span of an implant. Titanium and its alloys are the preferred metal materials for bone reconstruction in orthopaedics and dentistry because of their properties such as immunity to corrosion, bio-compatibility, strength, low modulus and density. To improve their performance various coatings are applied. Phospholipid coatings on titanium surfaces have been intensively studied in terms of adhesion, proliferation and differentiation of human bone derived cells, human chondrocytes, human mesenchymal stem cells and of macrophage stimulation. They proved to be a positive factor for cell-implant interactions. The study of the structure of the phospholipid multilayer under liquid conditions in presence of growth medium and Human Serum Albumin (HSA), which is the favorable condition for cell adhesion, will deliver key parameters to understand the interaction between cells and lipid coated implants.

Introduction

Permanent implants such as metallic hip prostheses are widely used and successfully implemented in medicine. The efforts are manifold to develop new implant surface modifications which may prolong the life span of an implant, or shorten the recovery time of a patient by faster and more stable implant incorporation. Titanium and its alloys are the preferred metal materials for bone reconstruction in orthopaedics and dentistry. To improve their performance various coatings are applied. The approach we follow is the biomimetic coating by phospholipids. By applying lipid bilayers on a metal surface we offer a liquid crystalline coating which should resembles a model cell membrane. We could prove that in the presence of lipids chondrocytes as well as osteoblasts or mesenchymal stem cells produce up to 30% more matrix protein which is important for the proper function of the tissue. In the present work we want to elucidate the reason for the improved protein production.

Materials and Methods

In this experiment we examined the coating by POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine) which was obtained by drying stacks of lipid bilayers on a titanium coated silicon block. A 1 mM chloroform/methanol (8:2) solution was spread by a nebulizer on the titanium surface (0.5 Bar nitrogen) followed by two minutes of drying under the same flow of nitrogen. After that the organic solvent was evaporated this system was measured in D_2O and D_2O based growth medium (h-GM or d-GM) with deuterated Human Serum Albumin (h-HSA and d-HSA). d-HSA was produced by DLAB. For d-GM, growth medium powder was solved in D_2O (0.02g per 1 mL). The influences of growth medium with the protein were investigated on freshly prepared POPE multilayer structure. All samples were equilibrated for at least 2 h in liquid environment. Moreover, the temperature behavior of the spray-coated sample was probed in the range from 20 °C to 42 °C.

The experiment setup includes three incoming angles (0.624°, 3 ° and 4.5 °) were used to obtain a q-range from 0.0045 to ~ 0.42 $Å^{-1}$. The wavelength resolution was 4.2%. Using of 2D detector gave us opportunity to collect off-specular scattering data.

Experimental Results

According to the measurement of the spray-coated titanium crystal at the lowest incident angle (0.624°) against D_2O, the two critical edges are clearly defined on the reflectivity curve (1). The observed drop of the intensity can be explained due to the attenuation of the beam, which passes through the thick POPE coating. Such a drop has be mentioned in literature by Zarbakhch in his neutron reflectivity investigation of 1-2 μm thick oil layers placed in between a silicon crystal and a D_2O interface [1].

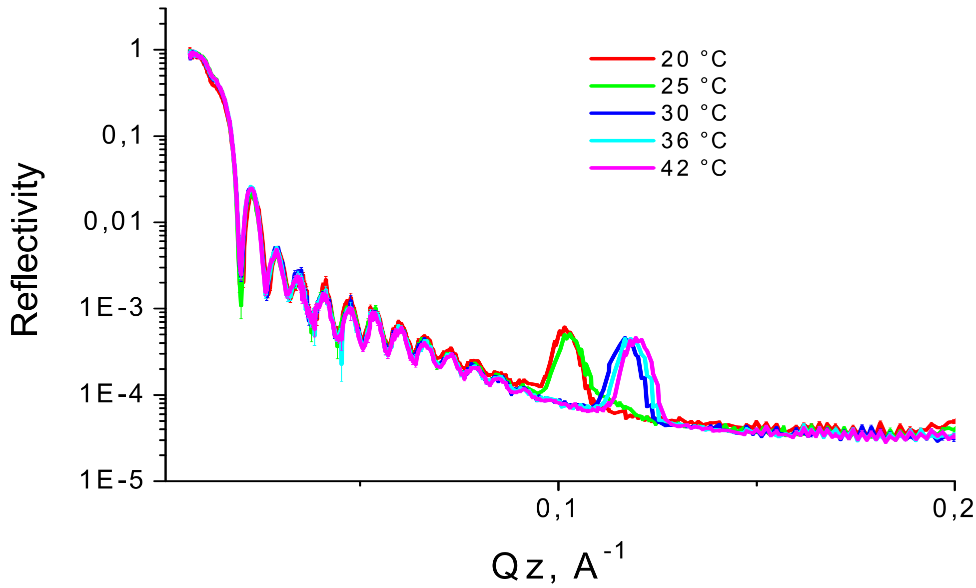

Figure1. Neutron reflectivity curves – the temperature behavior measured in D_2O.

The shape and positions of the oscillations remain unaffected for all chosen temperatures, meantime the Bragg peak shifts to the region of higher Q_z with the temperature increase. At the temperature 25 °C POPE lipid has a phase transition from the gel phase to the liquid crystalline phase due to melting of the hydrocarbon region of bilayers. The effect of the face transition is seen on the green specular reflectivity curve in Figure 1 as the double Bragg peak, means that both phases are present in the POPE multilayer stack.

For each applied temperature, the position of the Bragg peak is defined from the fit by a Lorentzian function. In that way it is possible to obtain the temperature dependence of the POPE d-spacing (see Figure 2). It is clearly seen that after the phase transition temperature the d-spacing of POPE bilayers changes linearly with temperature. Such a linear behavior for the POPE bilayer thickness has been observed from the X-ray diffraction experiment by Rappolt [2], however, in the case of the diffraction experiment, the POPE multilayer stack was not in contact with liquid as it is performed in our experiment. From the literature [3] it is known that the decrease of the POPE bilayer thickness with increase temperature is caused by continuous formation of trans-gauche rotamers within the alkyl chains.

The effect of the D_2O based growth medium with dHSA was detected by the shift of the linear temperature behavior of the POPE bilayer repeat distance (see Figure 2).

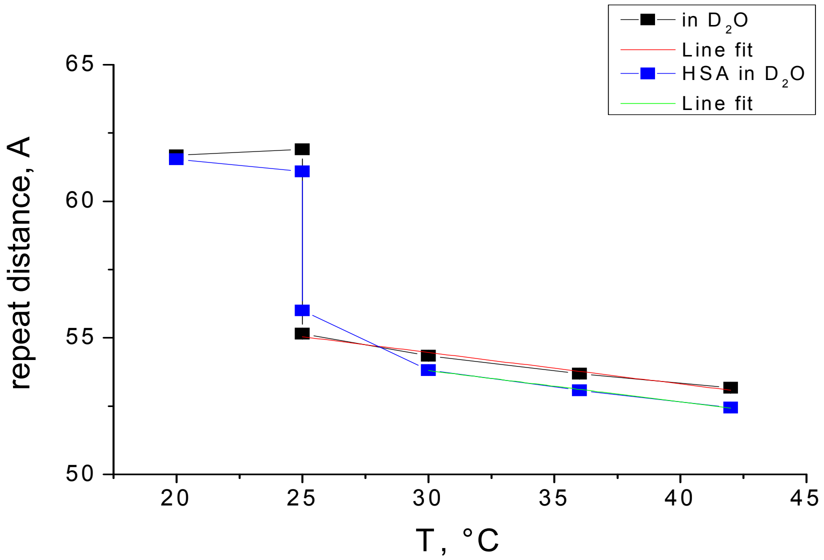

Figure 1. Temperature behavior of the Bragg peak position: 1) Black dots correspond to the d-spacing of the POPE bilayer at different temperatures in D_2O; 2) Blue dots - D_2O based growth medium with dHSA. 3) The red line and the green line – linear fit.

The shift is equal to 0.7 Å that is higher than possible mistake of the repeat distance determination, which is about 0.2 Å for the analysis of the reflectivity curves. The shift might take place due to the change of Ph of the contrast solution with adding growth medium powder.

References:

1. Zarbakhsh A., et al., *New Approach for Measuring Neutron Reflection from a Liquid/Liquid Interface.* Meas. Sci. Technol., 1999. **10**.
2. Rappolt M., et al., *Structure and Elasticity of Phospholipid Bilayers in the Lα phase: a Comparison of Phosphatilylcholine and Phosphatidylethanolamine Membrane.* Recent Research Developments in Biophiscs, 2004. **3**.
3. Rappolt M., et al., *Flexibility and Structure of Fluid Bilayer Interfaces.* Structure and Dynamics of Membranous Interfaces, 2008.