Experimental report

08/10/2015

Proposal: 9-12-367 **Council:** 10/2014

Title: Interaction between functionalizedFe3O4 nanoparticles and lipid bilayers

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Cholesterol, phospholipids, Fe3O4 nanoparticles

Instrument	Requested days	Allocated days	From	To
FIGARO	4	0		
D17	4	3	10/07/2015	13/07/2015

Abstract:

Recently Fe3O4 nanoparticles have been introduced as novel contrast agents for Magnetic Resonance Imaging (MRI) technique, with the potentiality of being at same time nanocarriers for drugs. However, there are still several concerns about nanoparticle biocompatibility, which is essential for both the application as contrast agents and drug delivery devices. Thus, we introduced a novel nanoparticle functionalization approach, based on the use of phospholipids, aiming to produce biocompatible functionalized Fe3O4 nanoparticles. In the process of the development of a biocompatible nanostructured system, it is important to consider how it interacts with the most external cellular component, the cellular membrane. With this focus, we propose Neutron Reflectivity (NR) measurements to study the interaction of functionalized Fe3O4 nanoparticles and lipid bilayers that will simulate a biological membrane. In the presence of interaction, the stability of the membrane with time will be evaluated. Finally, the interaction site within the membrane bilayer will be determined.

Interaction between Fe_3O_4 Nanoparticles and lipid bilayers

Introduction.

The Superparamagnetic Iron oxide Nanoparticles (SPIONs) have been proposed as Magnetic Resonance Imaging (MRI) contrast agents due to their property of reducing the transversal proton relaxation time. However, there are still several concerns about nanoparticle biocompatibility, which is essential for both applications as contrast agents and drug delivery devices.[1] Thus, we introduced a novel nanoparticle functionalization approach, which is based on the use of a phosphocholine, aiming at producing highly biocompatible SPIONs.[2] We further exploited the versatility of our functionalization strategy by successfully introducing on SPIONs surface an amphiphilic ruthenium complex, named ToThyCholRu, as an antitumoral drugs. In the process of development of a biocompatible nanostructured system, it is important to consider how it interacts with the most external cellular component, the cellular membrane. Recently we have performed Neutron Reflectometry (NR) measurements to study the interaction of phoshocholine-functionalized SPIONs and lipid bilayers that simulated a biological membrane. We demonstrated that the interaction between the SPIONs and the lipid bilayers was mostly superficial. Here we report the NR results on the characterization of the interaction between the SPIONs functionalized with the ToThyCholRu, named ToThyCholRu-SPIONs, and lipid bilayers containing different cholesterol concentrations.

Experimental section.

Neutron reflectivity measurements were performed on the D17 reflectometer. The first set of data was collected in order to characterize the lipid bilayers containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG) and cholesterol (CHOL) with composition POPC/POPG (90:10 w/w), POPC/POPG/CHOL (70/10/20 w/w) and POPC/POPG/CHOL (60:10:30 w/w).

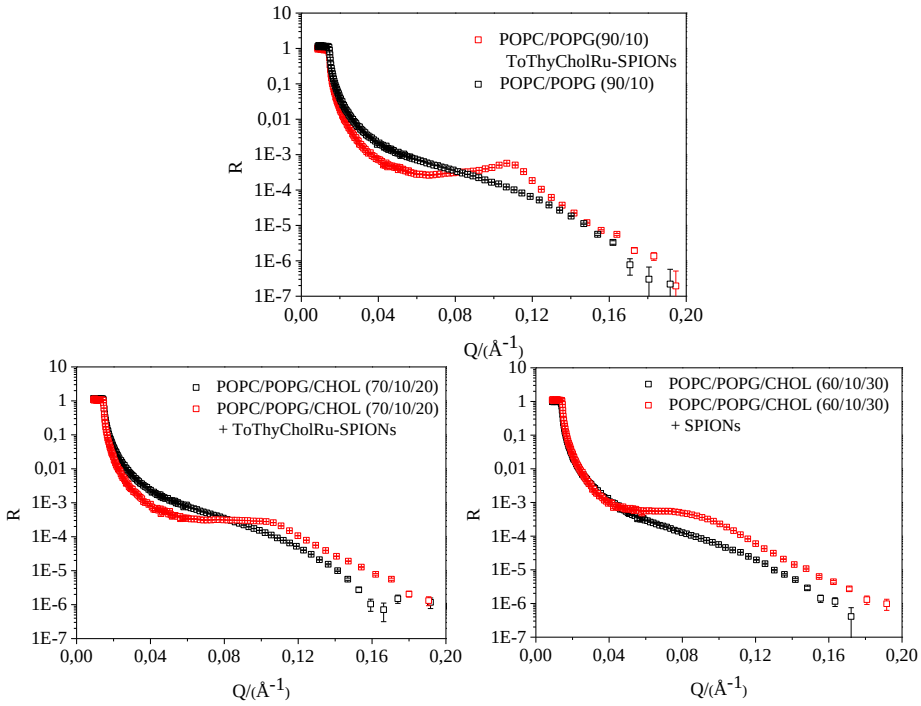

Figure 1: *Neutron reflectivity experimental data collected in D_2O POPC/POPG (90/10 w/w)+ToThyCholRu-SPIONs, POPC/POPG/CHOL (70/10/20 w/w)+ToThyCholRu SPIONs and POPC/POPG/CHOL (60/10/30 w/w)+ToThyCholRu-SPIONs, as reported in the legend. The collected data are compared with the ones corresponding to the pure bilayers*

The bilayers were prepared through vesicle adsorption on a silicon support. In particular, single unilamellar vesicle suspensions in D_2O were prepared at 0.5mg/ml total lipid concentration, and injected into the NR cells. After 30 min, the lipid excess was washed out by pumping D_2O into the cells. [3,4] The subsequent NR

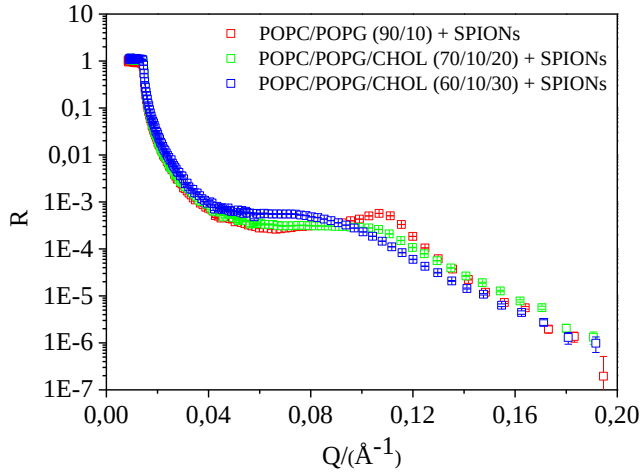

Figure 2: *Comparison between the collected reflectivity curves, corresponding to the investigated samples as reported in the legend.*

measurements, performed using three different contrasts (D_2O, H_2O and Silicon Match Water (SMW)), confirmed the formation of a single bilayer on the silicon surface.

After the characterization of the two bilayers, the D_2O suspension of ToThyCholRu-SPIONs was injected. For each contrast NR measurements were performed right after SPIONs injection, after 6h, and after several washing steps, in order to test the stability of the interaction between the ToThyCholRu-SPIONs and the bilayers.

The reflectivity curves collected for the samples in D_2O are reported in figure 1. By comparing the reflectivity curves of the lipid bilayers with the ones collected after the ToThyCholRu-SPIONs injection, it was concluded that the nanoparticles affected lipid bilayer. Indeed, the reflectivity curve shape resulted to be changed and the presence of a peak was observed. A preliminary data analysis suggested that in all the analyzed samples the lipid bilayer is not removed by the SPIONs. An hydrated stack of SPIONs layers partially penetrating the bilayer might explain the presence of the structure peak positioned at about Q= 0.1 $Å^{-1}$, which corresponds to a distance of about 60 Å compatible with the typical SPIONs size.

The different cholesterol concentrations in the lipid bilayers influenced the interaction with the ToThyCholRu-SPIONs. By increasing the cholesterol content the peak diagnostic of the SPIONs organization on the bilayer surface was reduced in terms of intensity and apparently shifted toward smaller Q values. We postulated that the reduction of intensity might be connected to a less dense stack of SPIONs on the bilayer surface ascribable to the higher rigidity of the lipid bilayer induced by cholesterol. However, a more detailed data analysis will confirm this hypothesis.

Conclusions.

Neutron reflectivity measurements were performed to investigate the interaction between the ToThyCholRu-SPIONs and lipid bilayers. The reflectivity curves show strong differences upon SPIONs injection with respect to the ones collected for the pure bilayers. We believe that this differences can be associated to the formation of a stack of SPIONs layers on the bilayer surface. Our preliminary data analysis suggests that the lipid bilayer is not removed by the ToThyCholRu-SPIONs. The organization of the SPIONs on the bilayer surface seems also to be affected by the rigidity of lipid bilayers induced by the presence of cholesterol.

References.

[1] Yu, M. K.; Park, J.; Jon, S. Targeting Strategies for Multifunctional Nanoparticles in Cancer Imaging and Therapy. *Theranostics* **2012,** *2* (1), 3-44.

[2] Luchini, A.; Vitiello, G.; Rossi, F.; De Ballesteros, O. R.; Radulescu, A.; D'Errico, G.; Montesarchio, D.; Fernandez, C. D.; Paduano, L. Developing functionalized Fe3O4-Au nanoparticles: a physico-chemical insight. *Phys Chem Chem Phys* **2015,** *17* (8), 6087-6097.

[3] Vitiello, G., Cholesterol modulates the fusogenic activity of a membranotropic domain of the FIV glycoprotein gp36, *Soft Matter*, 2013,9, 6442-6456.

[4] Merlino, A., Destabilization of lipid membranes by a peptide derived from glycoprotein gp36 of feline immunodeficiency virus: a combined molecular dynamics/experimental study, Journal of Phisical Chemistry, B, 2012, 116(1), 401-412.