MP-SPR measurements of soft and hard corona on nanoparticle in 100% serum

When nanocarriers are introduced into the blood circulation, they are rapidly covered with a protein corona, which is a complex layer of biomolecules such as proteins, lipids, and other plasma components. Further cellular responses to the nanoparticle depend on the composition of corona. Interaction of lipid based nanocarriers with 100% serum was studied using Multi-Parametric Surface Plasmon Resonance (MP-SPR). Protein corona formation on a nanocarrier surface was studied without disturbing its dynamics. Positively charged liposomes with oligo guanidyl lipid derivative (OGD) and replicas of the Doxil© without doxorubicin (DOX) were studied with and without PEGylation. Thickness and refractive index of lipid and corona layers were determined. The thickness of soft corona on OGD liposomes was 34.2 nm, whereas hard corona thickness was 9.6 nm.

Exploiting PureKinetics™ feature makes MP-SPR measurements suitable even in 100% serum samples but also for measurements in other complex liquids such as saliva (Sonny et al., 2010), milk (Ye et al., 2016) or sea water (Ma et al., 2015).

Introduction

Biomolecular interactions are routinely measured in the fields of drug discovery and biosensor development. Surface Plasmon Resonance (SPR) is well established method to measure label-free molecular interactions in real-time, but typically is limited only to 10% serum, which is not sufficiently mimicking the *in vivo* environment. A unique Multi-Parametric Surface Plasmon Resonance (MP-SPR) instrument can perform measurements in a wide angular range (40-78 degrees) and at more than one wavelength, which extends applicability of SPR also to complex liquids, and to nanoparticle and cell studies.

MP-SPR measures molecular adsorption in real-time and the same measurement also allows layer thicknesses to be calculated. In addition, MP-SPR Navi™ instrument fluidics is easily adjustable for nanoparticles and crude sample studies while still maintaining clogging-free-operation.

In SPR, crude samples usually produce big bulk (solvent) effects. In order to reveal binding, bulk correction is required. In traditional SPR, correction of large bulk signal is challenging and often impossible. The unique optical setup of the MP-SPR instruments enables simultaneous measurement of multiple optical parameters. Real-time cross-correlation of the parameters allows simple in line correction of the interfering bulk signal using the PureKinetics™ feature.

In drug discovery, complex media studies shorten the gap between *in vitro* experiments and further pre-clinical studies, whereas, in biosensors development the transitions from development to final product is simplified.

Materials and methods

Gold sensor slides coated with a 6-kD carboxymethyl-dextran hydrogel layer and functionalized with dodecyl lipid anchors were used to immobilize liposomes on a surface (Figure 1). Positively charged liposomes with oligo-guanidyl lipid derivative (OGD) and replicas of the Doxil© without doxorubicin (DOX) were studied. Liposomes with and without PEG were studied, after immobilization OGD liposomes were PEGylated *in situ* using polyanionic-PEG block-copolymer, whereas DOX liposomes were PEGylated *ex situ*. Sensor slide were used repeatedly after following cleaning steps: Hellmanex II 2 % or CHAPS 20mM, ethanol 80% and de-ionized water.

Blood drawn from seven healthy and fasted donors were used. Blood was left to clot, followed by centrifugation and collection of serum fractions before measuring 100% serum interaction with immobilized liposomes. SPR measurements were performed with MP-SPR Navi™ 200–L instrument at 20 °C using 100µL/min flow-rate. Full SPR curves were modelled using LayerSolver™ software to calculate layer thickness and refractive indices.

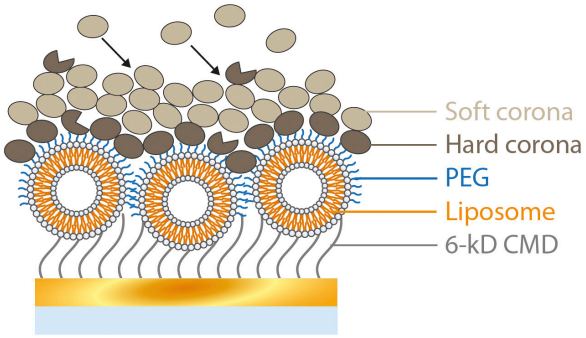

Figure 1. 100% serum sample interaction on liposomes was studied using MP-SPR. Liposomes with oligo-guanidyl lipid derivative (OGD) and replicas of the Doxil© without doxorubicin (DOX) were studied with and without PEGylation. Serum sample formed hard and soft coronas on the liposomes.

Results and discussion

Liposomes were successfully immobilized on a sensor surface. The formed layer thickness of OGD liposomes was 40.4 ± 9.3 nm, whereas PEGylated OGD liposome layers were 44.4 ± 4.2 nm thick. Refractive indexes were 1.35302 ± 0.0021 and 1.35679 ± 0.00292 respectively. All results are averages (±standard deviation, STD) of three independent measurements.

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To mimic blood flow conditions, interaction of serum samples with liposomes were studied under dynamic flow conditions at a controlled temperature (Figure 2).

Serum was found to form soft and hard coronas on a liposome surface (Figure 3). Soft corona refers to loosely bonded organic material on the nanoparticle surface or molecules that have weak interaction with the hard corona. On OGD + PEG liposomes the thickness of the soft corona was 22.2 ± 14.5 nm. Soft corona could be rinsed off with a flow of buffer, whereas 4.6 ± 2.7 nm thick hard corona stayed on a surface after rinsing. It was found that on plain OGD liposomes a thicker (9.6 ± 1.0) and denser (1.41121 – 1.44627) hard corona layer was formed, compared to the pegylated liposome samples. Differences in protein corona formation are believed to be caused by a different surface lipophilicity or hydrophilicity, surface charge and rheological properties.

The corona composition will vary also in dependence on the *in vivo* delivery route of the nanoparticle, such as subcutaneous, inhalation, intraveneous.

Results were combined with the protein liposome interaction data from MP-SPR. Findings indicated that OGD liposomes without PEG produced stronger surface-induced activation of the complement system (part of immune system) than PEGylated OGD liposomes. Further studies are required to confirm these findings.

Other research groups have exploited MP-SPR to study crude samples as well: Emilsson *et al*. (2015) used serum samples to measure the antifouling properties of polymers, Ma *et al*. (2015) studied marine antifouling using sea water, Sonny *et al*. (2010) studied cocaine detection from saliva samples, whereas Ye *et al*. (2016) studied antifouling of polymers using bovine milk.

Conclusions

Corona formation on a nanoparticle surface was studied in 100% serum sample for development of drug delivery nanocarriers. The robust fluidics and PureKinetics™ features of MP-SPR enable reliable results even in complex media. MP-SPR measures real-time interactions (affinity, kinetics, mass) and layer properties (thickness, refractive index), which makes instrument excellent for drug targeting and delivery studies, material characterization as well as biosensor development.

See also how to analyze dissociation kinetics of IgG from protein A using MP-SPR and PureKinetics™ (AN#147).

Original article:

Kari *et al*. Drug Delivery and Translational Research. 2016

References:

Emilsson *et al*., ACS Applied Materials & Interfaces, 7, 2015
Sonny *et al*., Proceedings of SPIE 7376, 2010
Ma *et al*., Langmuir, 31, 2015
Ye *et al*., Journal of Materials Chemistry B, 4, 2016

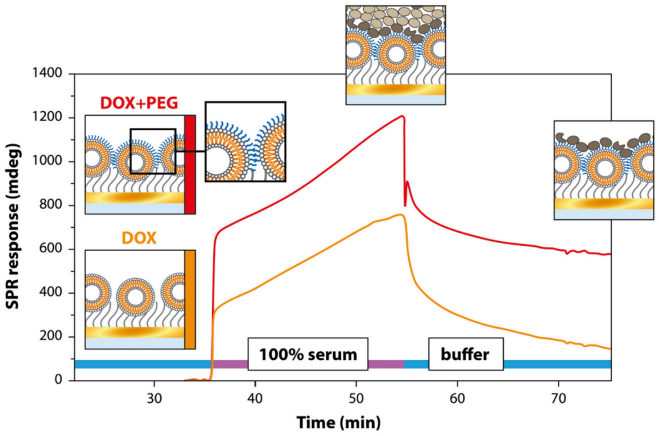

Figure 2. Sensogram during serum interaction on the DOX liposomes (replicas of Doxil© liposomes without doxorubicin). Based on layer thickness and refractive index information, a thinner soft corona was formed on PEGylated liposomes, whereas hard corona was thicker when compared to liposomes without PEGylation.

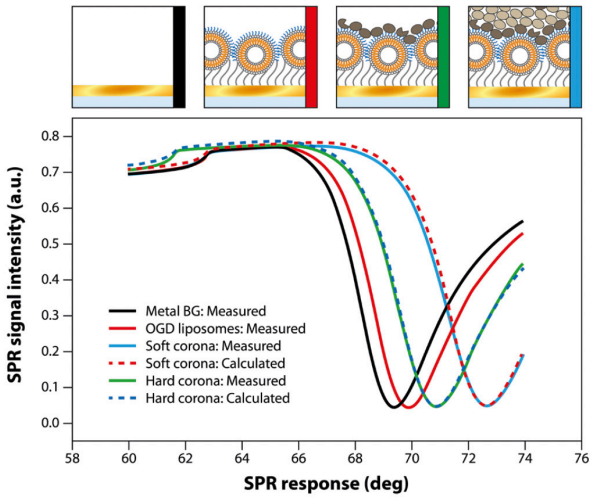

Figure 4. Full SPR curve during the oligo-guanidyl lipid derivative (OGD) liposomes deposition and serum interaction experiment. Black curve is a blank gold sensor slide, red curve surface with immobilized liposomes, blue curve soft corona and green curve is hard corona, which is left on a surface after rinsing with buffer. Dashed lines shows the fits for corona layers from LayerSolver™ thickness and refractive index analysis.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 210A VASA or 220A NAALI with additional wavelength -L

Sensor surface: Au, other metal or inorganic coating

Software: MP-SPR Navi™ Controller, DataViewer, LayerSolver™ and TraceDrawer™ for MP-SPR Navi™

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