Application Note # 152

Drug Delivery Nanocarrier Studies Using MP-SPR

Nanocarriers are being extensively studied as drug delivery systems for cancer and gene therapy amongst others. Block copolymeric micelles (LMP) were developed as mucoadhesive ocular drug delivery vehicles. Micelles and chitosan interaction parameters on mucin surface were determined using Multi-Parametric Surface Plasmon Resonance (MP-SPR). Micelles adhesion was found to increase slightly with increasing phenylboronic acid (PBA) content of the nanoparticle.

In a separate study, binding of C3b molecules to liposomes was measured using MP-SPR. C3b is part of the innate immune system and it serves as an opsonizing agent enhancing phagocytosis (more specifically tagging the cell for phagocytosis). The affinity of C3b on PEGylated oligo-guanidyl lipid modified (OGP + PEG) liposomes was $K_D = 7.2 \times 10^{-8}$ *M and surface mass density* $\Gamma = 120 - 200$ *ng/cm².*
The binding level was higher on liposomes without PEG.

Introduction

Clinical safety of the nanocarriers can be improved by decreasing their immunogenicity, or by increasing their cellular targeting ability. Nanocarriers are also widely optimized to improve drug delivery over tight biological barriers which are challenging to overcome, such as blood-brain and blood-ocular barrier.

Surface Plasmon Resonance (SPR) is a popular method to measure molecular interactions, such as drug and protein interactions in real-time and label-free. Multi-Parametric Surface Plasmon Resonance (MP-SPR) extends applicability of SPR from molecular interaction measurements also to more fundamental studies of nanoparticle/target and nanoparticle/cell interactions, including materials characterization.

The MP-SPR instruments can uniquely perform measurements in a wide angular scanning range (40-78 degrees) and at more than one wavelength, thus making it an valuable system for nanoparticle and cell studies. MP-SPR measures adsorption in real-time and the same measurement allows layer thicknesses to be calculated. In addition, the MP-SPR Navi™ instrument fluidics is easily adjustable for nanoparticle and crude sample studies while still maintaining blockage free operation.

The optical setup of the MP-SPR instruments enables measurement of multiple optical parameters simultaneously. Cross-correlation of the parameters allows simple in-line characterization of the interfering bulk signal using the PureKinetics™ feature and allows for real-time correction of the bulk. This is extremely useful when altering buffers are used, or when an optimal reference surface does not exist, such as during molecule or nanoparticle interactions with lipid layers or materials.

Materials and methods

Study I

A series of block copolymer micelles (LMP = poly(L -lactide)-b-poly(methacrylic acid-co-3-acrylamidophenylboronic acid)) was synthetized, containing increasing amounts of phenylboronic acid (LMP-0, LMP-5, LMP-10, LMP-20, and LMP-30). The size of liposomes, determined by dynamic light scattering, varied from 36 nm (LMP-0) to 64 nm (LMP-30). Liposomes and chitosan interaction on a mucin surface was measured for 50 minutes (Figure 1.A). Chitosan (1 mg/mL) interaction was measured as a positive control for muco-adhesion.

Cleaned gold sensor slides were incubated 24 hours in 100 µg/mL bovine submaxillary gland mucin (4 kDa) at 20 °C to form a mucin layer. SPR measurements were conducted with a SPR Navi™ 200 instrument in a simulated tear fluid (STF; 23.1mM KCl, 20.0mM $NaHCO_3$, 1mM $CaCl_2 \cdot 2H_2O$, 113.5mM NaCl; pH 7.4) using 50 µL/min flow-rate at 22°C. A more detailed description can be found in the original publication Prosperi-Porta *et al.* (2016).

Study II

Kari *et al.* (2016) measured the interaction kinetics of human native complement component C3b (180 kDa) to modified liposomes. A gold sensor slide coated with carboxymethyl dextran hydrogel (6 kDa) was functionalized with dodecyl lipid anchors, and then used to immobilize liposomes on the surface (Figure 1.B). Positively charged liposomes with oligo-guanidyl lipid derivation (OGD), with and without PEG, were studied. MP-SPR measurements were performed using SPR Navi™ 200–L instrument (at 670 nm and 785 nm wavelengths for each flow channel) at 20 °C using 100 µL/min flow-rate. Studied C3b concentrations were 0.00833, 0.0833, and 0.833 µM. The data was analyzed using TraceDrawer™ and LayerSolver™ software.

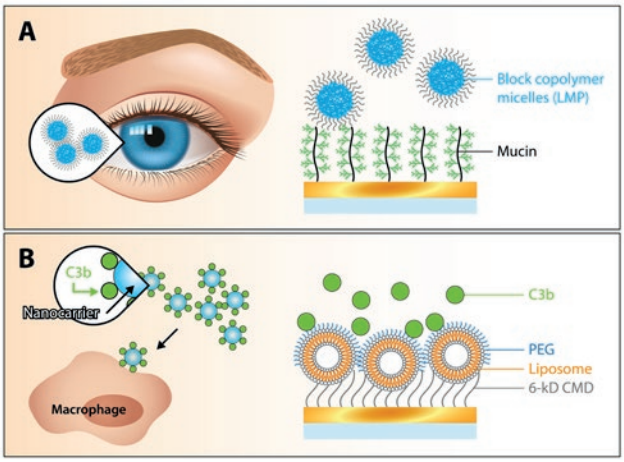

Figure 1. (A) Multi-Parametric Surface Plasmon Resonance (MP-SPR) measuring the binding of nanoparticles to surface immobilized molecules: Binding of block copolymer micelles (LMP) to mucin surfaces was studied for muco-adhesive ocular drug delivery. (B) Binding of molecules to surface attached nanoparticles: Binding of the opsonizing agent C3b to modified liposome-based nanoparticles was studied to assess nanocarrier immune response.

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Results and discussion

Study I

The main goal overall is to increase bioavailability of ocular drugs. Muco-adhesive nanocarriers were studied to improve ocular delivery of drugs. Muco-adhesion of the LMP micelles increased with increasing PBA content of nanoparticles, however, surface saturation appeared whereby additional PBA did not greatly increase muco-adhesion (Figure 2). Results indicate that higher PBA compositions may not be significantly more beneficial for clinical outcomes.

Study II

Immune responses towards lipid based nanocarriers were studied. A clearly larger MP-SPR response was detected in terms of C3b binding on OGD surface without PEG, compared to liposome surface with PEG (Figure 3). C3b surface mass densities (Γ) were ~410 – 680 ng/cm² on OGD liposomes without PEG, and Γ ~120 – 200 ng/cm² for PEGylated liposomes. The affinity of C3b on OGD liposomes was $K_D = 3.8 \times 10^{-8}$ M and clearly weaker binding was observed on OGD + PEG liposomes $K_D = 7.2 \times 10^{-8}$ M. Based on the results, OGD liposomes without PEG induced larger opsonization-related responses. This could lead to surface-induced activation of the complement system (part of immune system).

Additionally to interactions, lipid layer thickness and refractive index were determined using LayerSolver™ software. OGD liposomes layer thickness was 40.4 ± 9.3 nm, whereas PEGylated OGD liposomes layer was 44.4 ± 4.2 nm (Figure 4).

See also how MP-SPR measures uptake of silica nanoparticles by living cells (Baghirov *et al.* (2016), Suutari *et al.* (2016)) and enables to determine the mean size of surface bound nanoparticles (Rupert *et al.* (2016)).

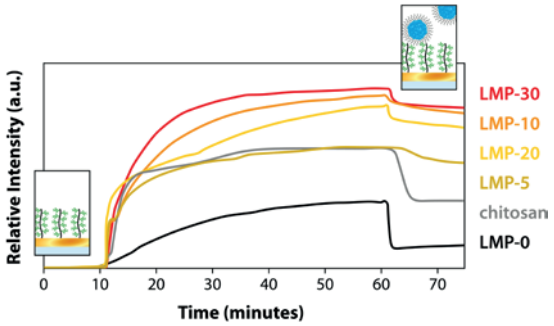

Figure 2. Binding of block copolymer micelles with varying phenylboronic acid (PBA) content (LMP-0, -5, -10, -20 and -30) to Mucin was measured. Muco-adhesion of the micelles increased with increasing PBA content of the nanoparticles and reached a plateau after LMP-10. Chitosan binding to mucin was used as a positive control for the binding. The measurements were performed in Fixed Angle mode of MP-SPR.

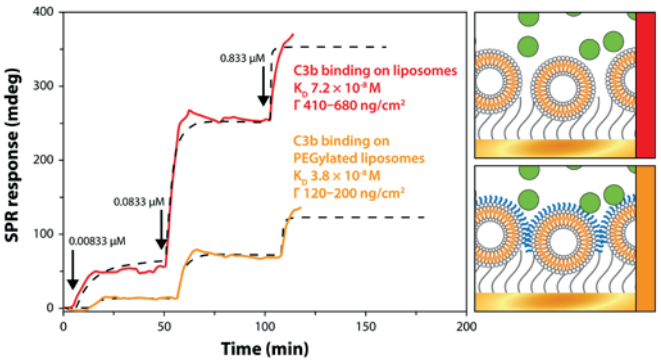

Figure 3. C3b binding to oligo-guanidyl lipid modified (OGD) liposomes. Binding was clearly stronger on liposomes without PEG. The measurement were performed in Angular Scan mode of MP-SPR.

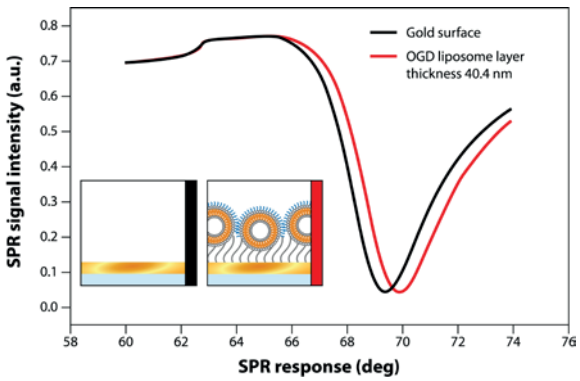

Figure 4. Measured full SPR curves before and after liposome layer deposition. Thickness (d) and refractive index (n) of the liposome layers were calculated using LayerSolver™.

Conclusions

MP-SPR Navi™ is an excellent instrument for nanocarrier development. It measures interactions of nanoparticles label-free and in real-time. MP-SPR also enables layer thickness and refractive index to be calculated, which can be used to ensure conformation of the particles on the surface.

The MP-SPR method is suitable to measure basically any material, hence metallic, polymeric, and lipid based nanoparticles can be studied. Assays with nanoparticles can be arranged so that they are attached to the sensor surface (Figure 1B) or that they float freely in the buffer stream above the modified sensor surface (Figure 1A), thus providing several options for assay development.

See how MP-SPR measures metal nanoparticles interactions (AN#140) or how MP-SPR is utilized to measure lipid layers (AN#139), interactions on living cells (AN#156) or drug release kinetics from polymer films (AN#141).

References

Original article:

Prosperi-Porta *et al.* Biomacromolecules, 2016, DOI: 10.1021/acs.biomac.6b00054

Kari *et al.* Drug Deliv. and Transl. Res. 2016, DOI 10.1007/s13346-016-0320-0

References:

Baghirov *et al.* PlosOne, 2016, 11(8), e0160705

Suutari *et al.* Small, 2016,

DOI: 10.1002/smll.201601815

Rupert *et al.* Anal. Chem., 2016, 88 (20), pp 9980–9988

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI, 210A VASA, or 200 OTSO

Sensor surfaces: Au, other metal or inorganic coating

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

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