Application Note # 174

Monitoring Nanoparticle-Biofilm Interactions Using MP-SPR

Bacterial biofilms contribute to persistent infections, posing a significant challenge in healthcare due to their resistance to antibiotics. Nanoparticles (NPs) have emerged as a promising approach for combating biofilm-related infections by either enhancing drug delivery or possessing intrinsic antibacterial properties. In this study, Multi-Parametric Surface Plasmon Resonance (MP-SPR) was utilized as a label-free, real-time analytical platform to monitor bacterial biofilm formation and evaluate interactions between biofilms and cerium oxide-coated mesoporous silica nanoparticles (CeO_2@MSNs) with different surface charges.

Introduction

MP-SPR instruments perform measurements uniquely in a wide angular range (40-78 degrees and captures the complete SPR curve). This enables the characterization of interactions between molecules and films, as well as the measurement of layer thicknesses and refractive indexes, ranging from the nanoscale to the microscale. In addition, the technique can utilize multiple laser wavelengths to determine interdependent optical parameters of layers during the same measurement. Furthermore, during an experiment, MP-SPR measures key parameters like Peak Angular Position (PAP), Peak Minimum Intensity (PMI), and Total Internal Reflection (TIR) angle (Figure 1). In this application note on bacterial biofilm formation and its interaction with nanoparticles (NPs), these parameters reveal mass accumulation on the surface and structural changes within the biofilm.

Materials and methods

Cerium oxide coated mesoporous silica nanoparticles (CeO_2@MSNs) were functionalized with polyethyleneimine (PEI), succinic anhydride (SUC), and acetic anhydride (ACA) to achieve positively, negatively, and neutrally charged NPs, respectively. Detailed information can be found in the original publication Mustafa *et al*., Smart Medicine 2023.

After initial cultivation the confluent bacterial biofilm of *Staphylococcus aureus* (suspended in PBS buffer) was seeded on a gold-coated sensor slide *in situ* in an MP-SPR instrument (equipped with 670 nm and 785 nm wavelength lasers in each flow channel) under continuous flow conditions (50 µL/min) and at 37 °C for 24 hours prior injections of NPs. Afterward, the NPs were immediately injected at a concentration of 100 µg/mL (dispersed in buffer) through the MP-SPR flow channels at 50 µL/min, exposing them to the biofilm for 48 hours.

The complete SPR curves were fitted with Fresnel transfer-matrix models and the dispersion relation at both laser wavelengths simultaneously to retrieve thicknesses and refractive indexes of the formed biofilm.

Results and discussion

MP-SPR successfully tracked biofilm growth, identifying distinct growth phases based on changes in complete SPR curve parameters (Peak Angular Position and Peak Minimum Intensity). Figure 2 illustrates the progressive increase in PAP over time, confirming bacterial adhesion, exponential growth, and maturation stages. The PMI signal peaked at the transition between exponential growth and maturation, indicating extracellular matrix production start, and then gradually decreased. This could be explained by an eventual uniform layer formation.

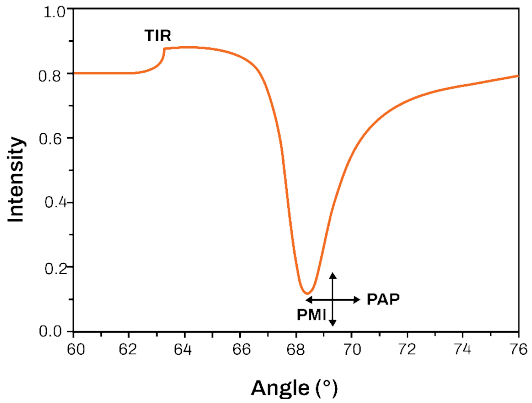

Figure 1.MP-SPR instruments measure complete SPR curve continuously, and multiple parameters can be followed in real-time, including Peak Angular Position (PAP), Peak Minimum Intensity (PMI) and Total Internal Reflection (TIR) angle.

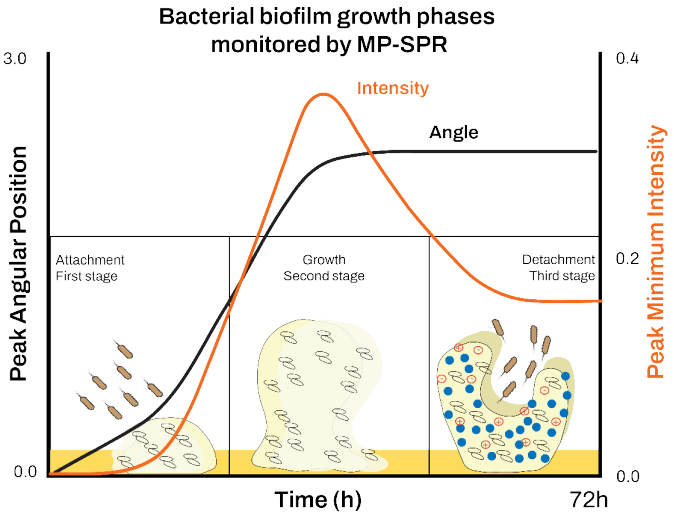

Figure 2. *In situ Staphylococcus aureus* bacterial biofilm growth phases monitored by MP-SPR (670 nm wavelength). The black and orange lines show the Peak Angular Position (PAP) and Peak Minimum intensity (PMI) changes plotted against time. ΔPMI indicates a stage after bacterial proliferation, which is due to production of extracellular matrix (ECM) and formation of a uniform layer.

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Upon NP exposure, it was seen that CeO_2@MSN-PEI induced the largest PAP shift (Figure 3), signifying strong biofilm interaction and penetration into the biofilm. In contrast, CeO_2@MSN-SUC and CeO_2@MSN-ACA caused minimal PAP changes, indicating weak interaction and low surface-level accumulation. These results align well with the confocal laser scanning microscopy data.

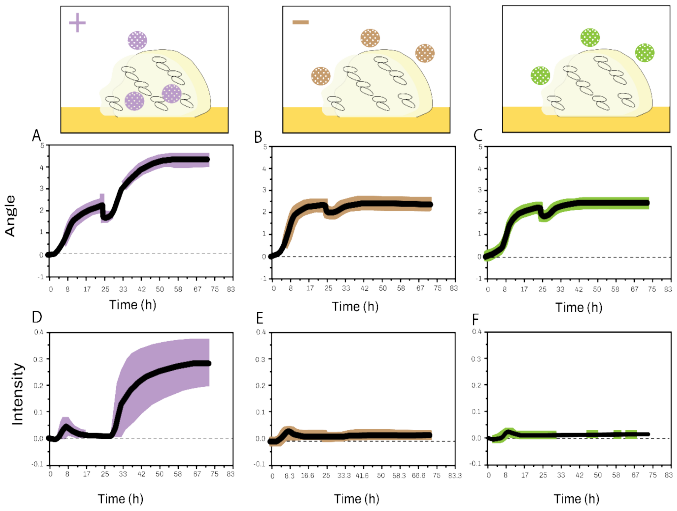

Figure 3. Average of real-time MP-SPR responses of the ΔPAP (upper graphs) and the ΔPMI (lower graphs) during *Staphylococcus aureus* bacterial biofilm growth and interaction of differently charged Nanoparticles (from t = 21.6 hours and onwards). A & D) Positively charged nanoparticles, B & E) Negatively charged SUC functionalized nanoparticles, C & F) Neutral ACA functionalized nanoparticles.

Fresnel modeling of MP-SPR data (Figure 4) provided quantitative measurements of biofilm (structured into layers for fitting purposes) thickness. Biofilm thickness before NP introduction was determined to be approximately 2.7 µm (with the refractive index of approximately 1.35). After, CeO_2@MSN-PEI exposure, some biofilm structural alterations could be identified, potentially causing the bottom part of the biofilm to lose a part of contact points with the sensor surface. These findings align with previously published work demonstrating that cationic NPs more effectively infiltrate biofilms due to electrostatic interactions with the negatively charged biofilm matrix [1].

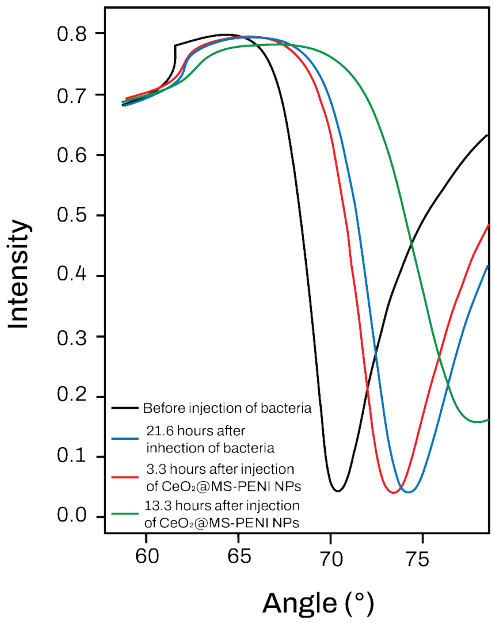

Figure 4. The unique, complete SPR curve measurement from MP-SPR allows tracking not only peak angle changes but also intensity parameters, which are crucial for this study. Complete MP-SPR curve (at 5 min) before the injection of bacteria (black), t = 21.6 h after the start of the injection of bacteria (blue), 3.3 h (red, time = 25h in Figure 3) and 13.3 h (green, t = 35 h in Figure 3) after the start of the injection of positively charged PEI coated nanoparticles.

MP-SPR provided real-time kinetic data on NP-biofilm interactions, revealing structural rearrangements in the biofilm upon NP exposure. Unlike traditional methods such as confocal microscopy (used as an alternative technique in the original publication) and flow cytometry, MP-SPR allowed continuous monitoring over 72 hours without the need for labeling, offering a powerful tool for biofilm research and antimicrobial NP development.

Conclusions

MP-SPR is a powerful, label-free platform for real-time monitoring of biofilm formation and nanoparticle (NP) interactions. MP-SPR captures uniquely the complete SPR curve and it multiple parameters, providing crucial insights into biofilm characteristics. Its ability to monitor biofilm growth over days and measure thickness using multi-wavelength analysis, along with its capability to work with layers ranging from nanometers to mictrometers, makes it uniquely suited for studying biofilm dynamics. This study underscores the role of NP surface charge in biofilm penetration, with positively charged CeO_2@MSN-PEI demonstrating superior interaction. With these capabilities, MP-SPR is an invaluable tool for advancing antibacterial strategies and biofilm research.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI or 210A VASA

Sensor surface: Au

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

Original publication:
Mustafa *et al*., Smart Medicine, 2023, 2(3).

References
1.Dong *et al*., PLoS One ,2015, 10, e0131806.

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