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Conjugation of Molecularly Imprinted Polymer Nanoparticles with Metal Nanoparticles for Signal Enhancement in QCM-based Sensor Applications

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Summary:
Solid phase synthesis of molecularly imprinted polymer nanoparticles (nanoMIPs) to detect the antibiotic vancomycin (VM) in a QCM-based assay format was established and optimized. To improve the limit of detection in gravimetric sensor setups, the nanoMIPs are designed to be further conjugated to metal nanoparticles for signal enhancement.

Keywords: QCM sensors, nanoMIPs, Signal Enhancement, Molecularly Imprinted Polymers, Biomimetic

Introduction

Molecular imprinting is a method used to obtain highly selective polymer materials that can offer some advantages over biological recognition units, such as an increased robustness against harsh, non-physiological conditions, long shelf life and reduced time and cost of production. Over the past decade, great focus has been put on nanosized imprinted particles (molecularly imprinted polymer nanoparticles, nanoMIPs) as promising materials for molecular recognition in the field of biosensors and assays. Obtained by a straightforward solid phase synthesis protocol, their high affinity, homogeneous binding sites and low non-specific binding yielded performances comparable to natural antibodies in ELISA, SPR and QCM-based assays.[1]–[3] However, limitations regarding achievable sensitivity in gravimetric detection are set by the low density of the polymer material.

This project focusses on the synthesis of nanoMIPs that can be conjugated to other, heavier nanoparticles (NPs), such as titanium dioxide-NPs. Binding of those nanoMIP-TiO_2NP conjugates would significantly increase the change of mass in a QCM-based sensor setup and therefore improve the limit of detection.

Methods

The solid phase synthesis of poly(n-isopropyl-acrylamide) (pNIPAM) based nanoparticles imprinted with the antibiotic vancomycin (VM) as a benchmark molecule was optimized. Special attention was paid to the choice of functional monomers. Here, not only the affinity towards the analyte, but also possible functional groups for further conjugation were considered when choosing the polymer composition. For that purpose, the functional monomer acrylic acid (AA) was added to the polymerization mixture of N-isopropyl-acrylamide (NIPAM) and N,N'-methylene-bis-acrylamide (BIS, crosslinker). For one, AA can introduce selective interaction sites with the analyte via hydrogen bonding. Moreover, it provides carboxylic groups within the polymer network, that can be used for further coupling via EDC/NHS crosslinking with amine-functionalized metal-NPs.

The nanoMIPs were characterized regarding their size, shape and polydispersity using dynamic light scattering and scanning electron microscopy. Fluorescence quenching and QCM-binding studies were used to assess the affinity between the analyte and the nanoMIPs as well as the nanoMIP crosslinking to an amine-bearing sensor surface.

Results

Fig. 1 shows both SEM and DLS characterization of pNIPAM-based nanoMIPs containing AA as functional monomer. Both SEM and DLS results indicate that the particles are of uniform,

spherical shape with an average diameter slightly below 100 nm. The PDI of 0.1 indicates a highly monodisperse size distribution of the nanoMIP suspension.

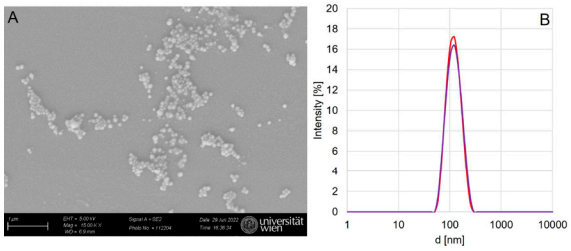

Fig. 1 A: SEM images of nanoMIPs, average diameter = 97.7 ± 0.5 nm, B: Size distribution of nanoMIP suspension in MQ H_2O measured by dynamic light scattering, d_h = 95.3 ± 15.4 nm, PDI = 0.1.

Fluorescence measurements show strong interaction between the imprinted particles and the analyte (Fig. 2). Mixing 250 ppm of VM with 25-250 ppm nanoMIPs results in concentration-dependent reduction of the VM-fluorescence by more than 80%; no such effect can be observed when adding non-imprinted reference particles with the same polymer composition. This indicates a significant increase in affinity between analyte and polymer due to imprinting.

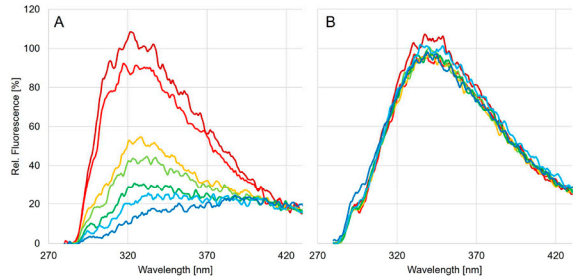

Fig. 2 Fluorescence signal of 250 ppm vancomycin in presence of 0-250 (red to blue) ppm of A) VM-imprinted nanoMIPs and B) non-imprinted reference NPs.

Affinity between the analyte and the nanoMIPs was also confirmed by QCM measurements (Fig. 3). A dual channel QCM was functionalized with VM on one electrode, while the other one was blocked with ethanolamine (EA). While the VM-functionalized channel shows a concentration-dependent frequency shift upon nanoMIP injection, the EA-electrode shows only small shifts at higher concentrations, indicating low non-specific binding of the nanoMIPs.

Lastly, QCM measurements to assess crosslinking between the carboxylic groups in the polymer network and an amine bearing compound were conducted. A cysteamine-functionalized QCM electrode was exposed to EDC/NHS activated nanoMIPs. As the measurement in Fig. 4 shows, the nanoMIPs bind to the QCM surface. Several washing steps with buffer did not remove the nanoMIPs, indicating covalent crosslinking to the cysteamine surface.

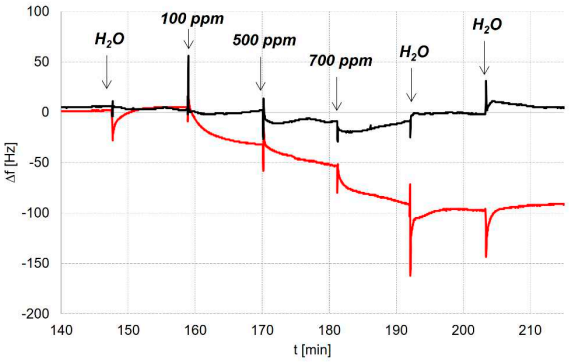

Fig. 3 Response towards varying nanoMIP concentrations of a dual channel QCM functionalized with VM on one channel (red) and ethanolamine on the other one (black).

As a next step, the nanoMIPs will be conjugated to APTES-functionalized TiO_2-NPs. The conjugates then can be used in a competitive assay format.

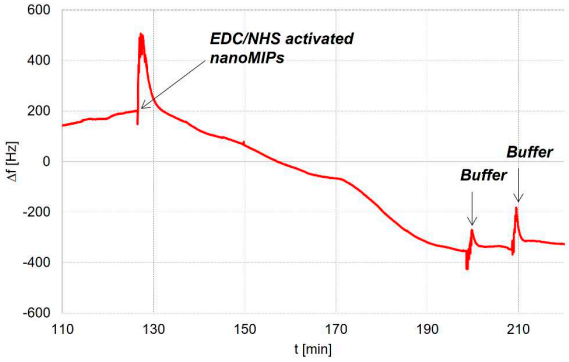

Fig. 4 Binding of AA-containing nanoMIPs activated by EDC/NHS to an amine-functionalized QCM electrode.

References

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