Surface Characterization of Escherichia Coli-Imprinted Polymers Using Confocal Raman Microscopy

Birgit Bräuer, Felix Thier, Marius Bittermann, Dieter Baurecht, Peter Lieberzeit

1 University of Vienna, Faculty of Chemistry, Department of Physical Chemistry
Währinger Straße 42, 1090 Vienna, Austria

Introduction
The Gram-negative bacterium Escherichia coli (E. coli) is considered an indicator of hygiene in food and water. Therefore, a variety of methods has been established for the detection, identification and quantification of this microorganism, most of which require growing the bacteria. In addition, available techniques such as flow cytometry depend on trained staff with substantial expertise (1). However, it is possible to generate E.coli-sensitive sensors based on E.coli-selective molecularly imprinted polymers (MIPs) as receptors on Quartz Crystal Microbalances (QCMs) for direct detection of the microorganism in water (2). In order to render the synthesis of the MIPs reproducible and to assess success of the imprinting, different techniques have been established to characterize MIPs, including Atomic Force and Optical Microscopy. Both provide topological information but no information about chemical composition of the surface. We employed Confocal Raman Microscopy for optical and chemical surface characterization of E.coli-MIPs (poly(styrene-co-divinylbenzene)) and assessment of success of the imprinting procedure. Furthermore, we combined Confocal Raman Microscopy with Partial Least Squares Discriminant Analysis (PLS-DA) to distinguish different bacteria species on the E.coli-MIP for further selectivity studies.

E. coli-Imprinted Polymers

Confocal Raman Microscopy and PLS-DA

Results

Fig. 1-2. AFM and Raman images of untreated (1B, 1C) and E.coli-treated MIPs (2B, 2C). AFM measurements confirmed the imprinting of E.coli on poly(styrene-co-divinylbenzene) (1B) and the presence of E.coli on the E.coli-treated MIP (2B). Overlaying of images of the Raman intensity at 2908 cm⁻¹ using WITec Raman TV obtained from the untreated (1C) and E.coli-treated MIP (2C) with the corresponding white light images (1A and 2A) showed that Confocal Raman Microscopy allowed for straightforward differentiation between imprints, polymer and E.coli. The accuracy of this differentiation was also confirmed by AFM scans on the exact same sample area in the case of E.coli-treated MIP (2B). WITec True Component Analysis of Raman images scans of E.coli-treated MIP yielded 2 components: E.coli attached to poly(styrene-co-divinylbenzene) (1D) and plain poly(styrene-co-divinylbenzene). Subtraction of the plain polymer component from the other one yielded a spectrum similar to a clean E.coli spectrum (2D). This confirmed the presence and location of E.coli on the MIP suggested by the AFM and simple Raman intensity images at 2908 cm⁻¹.

Raman imaging parameters: 532 nm laser wavelength, 8 mW laser power, 0.1 s integration time per spectrum, all images and spectra acquired on a WITec alpha 300 system.

Fig. 3: Single spectra of E.coli and L.lactis were acquired on E.coli-imprinted poly(styrene-co-divinylbenzene) at the locations indicated in 3A (E.coli) and 3B (L.lactis). The spectra were preprocessed [baseline correction, normalization and autoscaling] and a PLS-DA model with one latent variable was established to successfully differentiate between the two bacteria species on the polymer surface (3C). Raman parameters for single spectra: 532 nm laser wavelength, 8mW laser power, 3x20s integration time.

Conclusion
Confocal Raman Microscopy was successfully used for the assessment of imprinting success in E.coli-imprinted poly(styrene-co-divinylbenzene). Furthermore, different bacteria species, E.coli and L.lactis, could be differentiated on the surface of the MIP using a combination of Confocal Raman Microscopy and Partial Least Squares Discriminant Analysis, which provides a basis for further selectivity studies of the polymer.

References