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

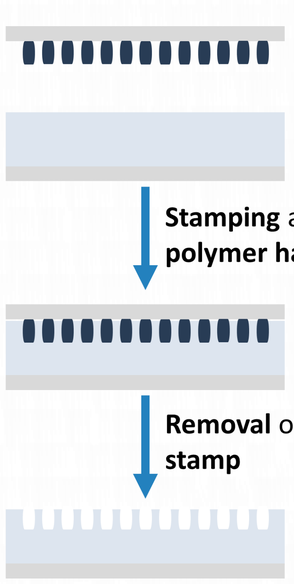
Birgit Bräuer[1], Felix Thier[1], Marius Bittermann[1], Dieter Baurecht[1], Peter Lieberzeit[1]

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

Introduction

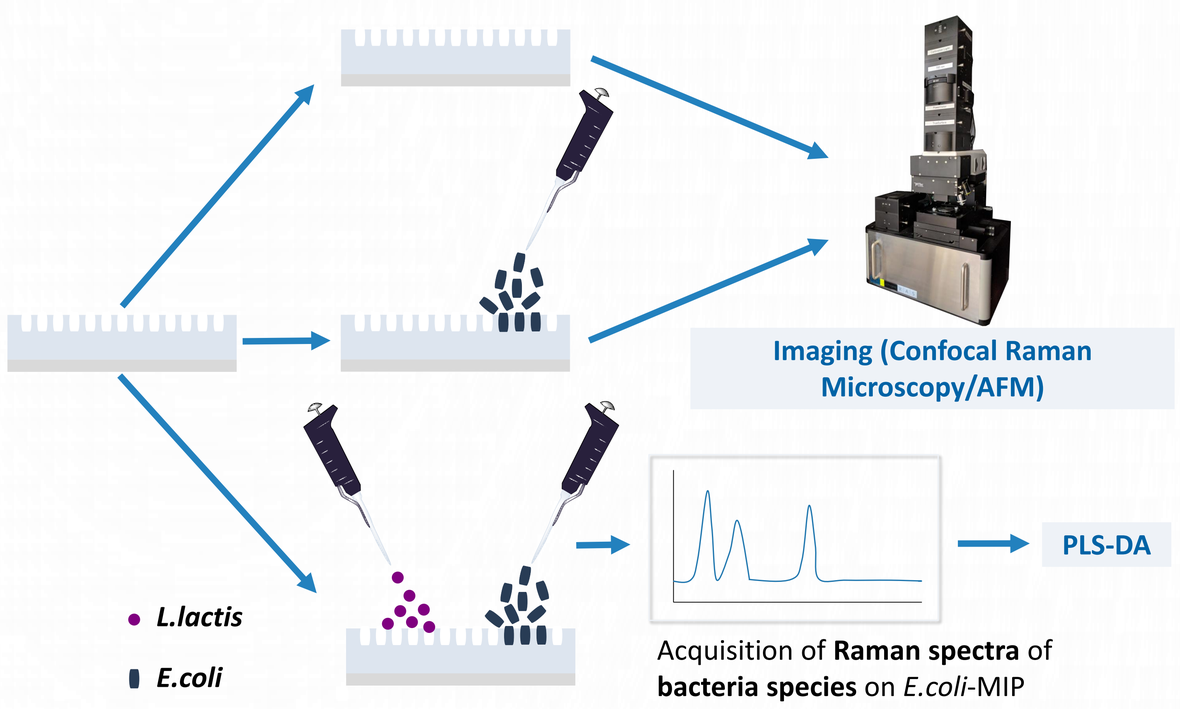
The Gram-negative bacterium *Escherichia coli* (*E. coli*) is considered an **indicator of hygiene** in food and water. Therefore, a variety of methods has been established for the **detection**, **identification** and **quantification** of this microorganism, most of which require growing the bacteria. 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



MIP should be **complementary** to the template (***E.coli***) regarding **shape** and orientation of **functional groups**

Confocal Raman Microscopy and PLS-DA



Acquisition of **Raman spectra** of **bacteria species** on *E.coli*-MIP

Results

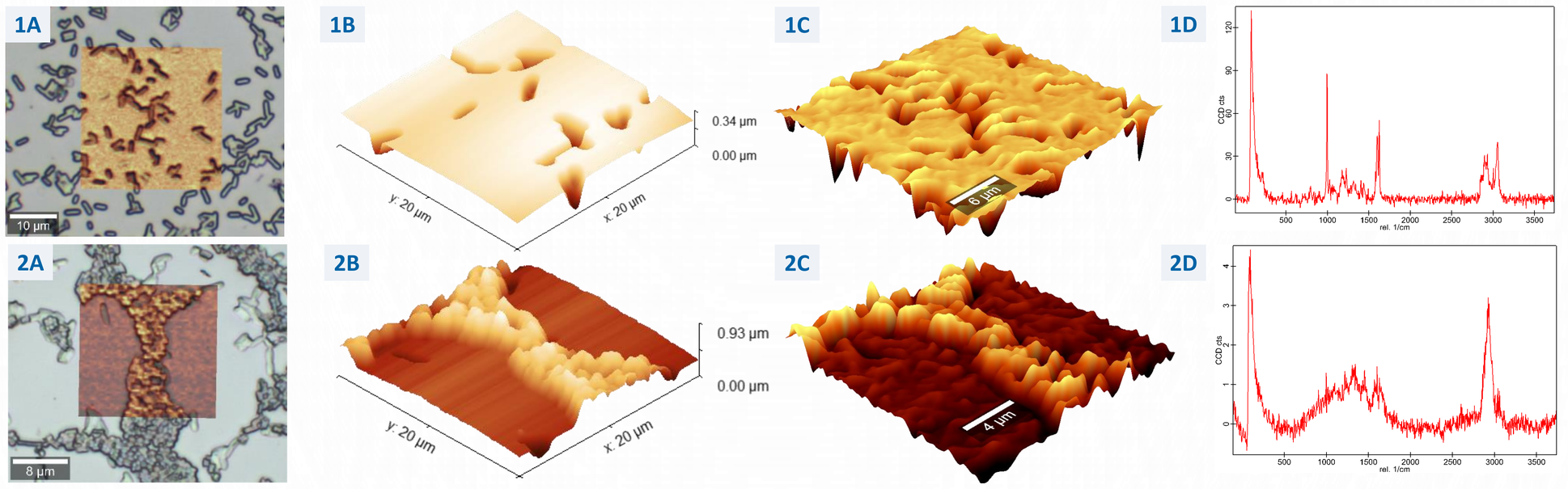


Fig. 1-2: **AFM** and **Raman** images of untreated (1B, 1C) and *E.coli*-treated MIPs (2B, 2C). AFM measurements **confirmed** the **imprinting of *E.coli*** on poly(styrene-co-divinylbenzene) (1B) and the presence of *E.coli* on the *E.coli*-treated MIP (2B). **Overlaying** of images of the **Raman 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 image 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

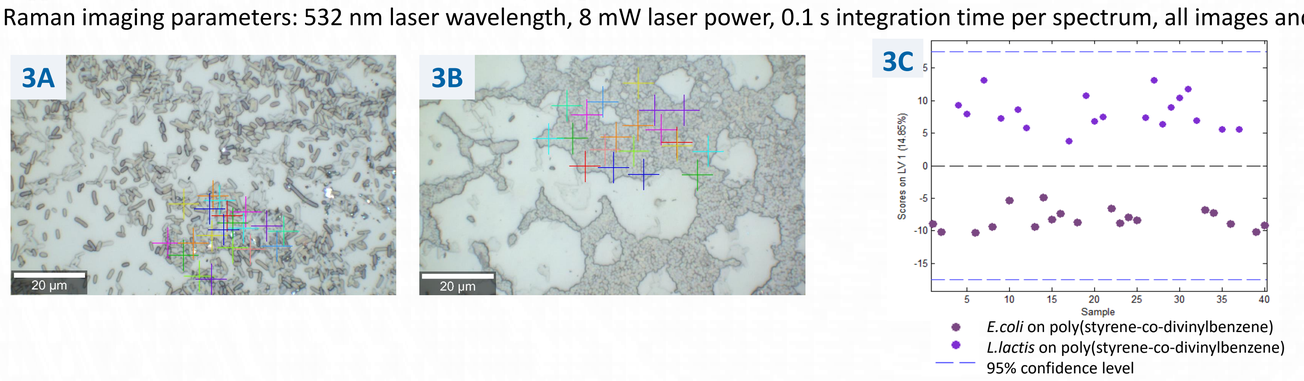


Fig. 3: Single spectra of ***E.coli*** and ***L.lactis*** were acquired on *E.coli*-imprinted poly(styrene-co-divinylbenzene) at the locations indicated in 3A (*E.coli*) and 3B (*L.lactis*). The spectra were preprocessed (baseline correction, normalization and autoscaling) and a **PLS-DA model** with 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

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2. Poller, Anna-Maria, et al. Surface Imprints: Advantageous Application of Ready2use Materials. ACS Applied Materials and Interfaces. 2017, Vol. 9, pp. 1129-1135.