Raman Microscopy and Surface-enhanced Raman Scattering (SERS) for in situ analysis of biofilm

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Biofilms represent the predominant form of microbial life in natural environment and can occur at nearly all interfaces (solid–liquid, solid–air, liquid–liquid, and liquid–air). They are communities of microorganisms embedded in a matrix of extracellular polymeric substances (EPS, such as polysaccharides, proteins, glycoproteins, nucleic acids, lipids, and humic-like substances). Depending on the biofilm type and microorganisms involved, up to 90% of the particulate fraction of the biofilm can be EPS [1]. Detailed information about chemical composition and structure of the EPS matrix is of great importance in medical, industrial and technological processes.

Raman microscopy (RM) is a capable tool that provides detailed chemical information about biological samples ("whole-organism fingerprints") with spatial resolution of optical microscopes. No or limited sample preparation and low water background make RM beneficial for in situ study of biofilms, since water is the major component of the biofilm matrix. We applied RM for the chemical characterization of different structures in a multispecies biofilm matrix, including microbial constituents/components and EPS. We demonstrated that RM can correlate variations of the chemical composition to structural appearances within the EPS matrix, and provides detailed chemical information about different constituents of a complex biofilm matrix [2]. The results of RM biofilm analysis are in good agreement with data obtained by confocal laser scanning microscopy [3].

The Raman signal can be significantly enhanced if the analyte molecule is attached or in the immediate proximity to metallic (Ag, Au or Cu) substrate with nanometer-roughened surface. This technique is known as surface-enhanced Raman scattering (SERS). The total enhancement factor due to electromagnetic ("localized surface plasmon resonance") and chemical enhancement ("charge transfer") is in the range of $10^3 - 10^6$, in some cases up to $\sim 10^{14}$ can be achieved [4]. Additionally, SERS has a fluorescence quenching effect. Thus, SERS overcomes the problem of limited sensitivity peculiar to normal RM. We employed colloidal silver nanoparticles [5] for in situ SERS measurements of biofilm by RM and obtained reproducible SERS spectra from different biofilm constituents [6]. The achieved enhancement factor of several orders of magnitude illustrated a good potential of SERS for sensitive chemical analysis of biofilms, including the detection of different components and the determination of their relative abundance in the complex biofilm matrix.

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