
Bacterial Adhesion Structures Studied by the Combination of Atomic Force and Fluorescence Microscopy

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The use of AFM in biology has increased tremendously in the last decade. Most applications of AFM in biology have studied model systems of well-described cell cultures or biomolecules. The biggest obstacle for applying AFM in more complex systems like natural microbial biofilms, is the challenge of identifying the observed cells and structures. The ability to combine AFM and optical microscopy allows the use of fluorescence labelling, and this opens up for studies of mixed microbial communities and uncultured organisms at the single-cell level with sub-cellular resolution.

In this study we combined AFM imaging with optical microscopy to investigate the structure, of extracellular biomolecules used for bacterial attachment and biofilm development. As a “proof of concept” we analysed pure cultures and natural biofilms from drinkingwater in different stages of the biofilm development. AFM imaging was combined with fluorescence *in situ* hybridisation (FISH) for bacterial identification and stains for protein, eDNA, polysaccharides and amyloid fibrils.

We found that microcolonies appearing after 3-5 days of biofilm development were surrounded by an amorphous matrix composed of polysaccharides, protein and DNA. This was not seen around single cells. While the polysaccharide matrix was present only in direct association with cells, eDNA could be seen across larger areas several microns away from cells or microcolonies, and it formed network-like structure. We find that AFM in combination with fluorescence imaging is a promising technique that provides detailed information about extracellular biomolecules, which furthers our understanding of the structure and function of adhesins, and their involvement in bacterial attachment and biofilm development in pure cultures as well as natural environments.