



## **Imaging mass spectrometry in the study of biofilm formation in a subsurface environment**

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Biofilms play a key role in biomineralization and rock weathering and consequently, in the interaction between the geo- and biospheres. Before a biofilm can evolve, individual microbial cells need to attach to a surface. The attachment of cells can be enhanced by thin films of organic molecules. Such so-called conditioning films form whenever a material surface is exposed in an aqueous medium (Donlan, 2002; Loeb & Neihof 1975). In this study, Time of Flight-Secondary Ion Mass Spectrometry (ToF-SIMS) and Scanning Electron Microscopy (SEM) were used to investigate the nature and architecture of conditioning films and the initial attachment of microbial cells to a pristine substrate exposed in a subsurface environment.

Experiments were conducted in the Äspö Hard Rock Laboratory (SE-Sweden), a 3.8 km long tunnel going down to a depth of 450 m bsl. Dark, airtight flow reactors simulating the environmental conditions in the fractures of the surrounding granodioritic rock were equipped with silicon wafers and connected to a subsurface aquifer. Samples taken after 10 min, 100 min, 1000 min, 10 d, 15 d, and 90 d, respectively, were directly analysed by ToF-SIMS. Short-chain hydrocarbon and oxygen-functionalised fragments revealed that different types of organic compounds adhered to the silicon surface already after a few minutes of exposure to the fluids. Mapping the lateral distribution of these compounds and comparison with the distribution of other, possibly contaminant-derived organic fragments indicated that the former indeed represent molecules of the initial conditioning film. The subsequent attachment of different cell types to the exposed surface was demonstrated with SEM, thus providing an insight into the modes of initial biofilm formation on fluid-exposed surfaces in a continental deep biosphere environment.

### References:

- Donlan, Rodney M., 2002. Biofilms: microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), 881–890.  
Loeb, George I. & Neihof, Rex A., 1975. Marine Conditioning Films. *Applied Chemistry at Protein Interfaces*, 145, 319-335.