X-ray Standing Wave Fluorescence for the Analysis of Bacterial Biofilms

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Introduction

Microbial biofilms play an important role in the pathogenesis of various human diseases, in many other natural systems, and in the performance of man-made devices that operate in those systems. Controlling biofilm growth requires an improved understanding of biofilm development, which in turn requires novel methods of chemically characterizing biofilms in situ [1]. Signaling species, many metabolites, antimicrobial agents, and substrate degradation products are molecular species often found within biofilms. Long period x-ray standing wave fluorescence (XSW) has been previously used to probe metal ions within intact microbial biofilms [2] and these strategies are described here for the detection of molecular analytes with biofilms. XSW can be used to determine the spatial resolution of a molecular species in the condensed phase with ~1 nm resolution. Chemical derivatization is used to permit selective detection of the analyte in situ, as shown previously when XSW was used to probe the conformation of a bromine labeled-polyethylene glycol-peptide construct (Br-PEG-peptide) adsorbed at the liquid-solid interface of a modified polystyrene surface [3].

Methods and Materials

Wild type *Bacillus subtilis* 168 biofilms are grown for several days in a Petri dish with LB media. The biofilms are then transferred to either Br-PEG-peptide or Br-tyrosine (Br-Y) coated polystyrene and cultured for ~3 hours. XSW measurements of the Br distribution are performed as previously described [3] except that the ChemMatCARS beamline is utilized at the Advanced Photon Source.

Results

Fluorescence background signal from the biofilm/polystyrene interface without added Br is low, indicating that the Br tag atom can be used to monitor low levels of Br-tagged adsorbate within the biofilm (data not shown). Experimental XSW results shown in Figure 1 are analyzed to determine the Br distribution by XSW as the Br-tagged analyte spread into the biofilm. However, fully dynamical treatment of x-ray scattering shows that the large thickness of the biofilms tightens the constraint upon the flatness of the biofilm-air interface if standing waves are to be observed. The biofilms studied apparently do not meet this flatness constrait and so no meaningful information on the Br spatial distribution within the biofilm could be extracted.

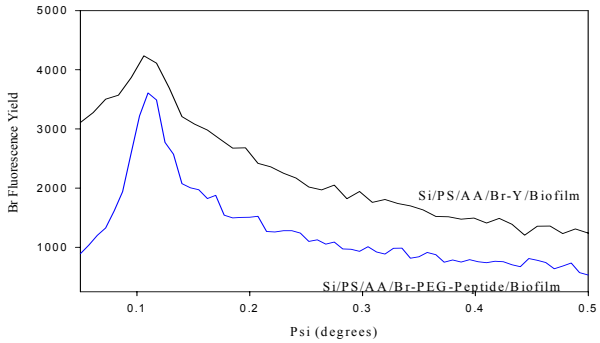

Figure 1: Br x-ray standing wave fluorescence (XSW) versus scattering from Br-PEG-peptide (bottom) and Br-Y (top) adsorbed on smooth polar surfaces, after *B. subtilis* biofilms have been cultured on those surfaces for ~3 hours.

Discussion

Br-derivatization shows high contrast within *B. subtilis* biofilms which do not naturally contain high levels of Br and therefore may be used to add contrast in x-ray fluorescence imaging experiments. However, XSW results on Si substrates cannot distinguish the position of the Br tag within the biofilm due to the large film thickness and the roughness of the air-biofilm interface. Simulations are underway to determine whether multilayer substrates improve the possibility of using XSW to study molecular transport into biofilms. The eventual hope is to use this strategy for studies of drug transport and metabolite detection in cells and tissue; in vitro and in vivo degradation of biomaterials and tissue engineered constructs; small molecule combinatorial arrays; and related problems.

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