NMR microscopy of dental biofilm metabolism

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Introduction. Dental caries – a biofilm infection - is one of the most prevalent and costly bacterial diseases in humans. Little is known about the metabolic mechanisms involved and organic acids produced (identities, concentrations, rates of production, and residence times) that are responsible for tooth-enamel demineralization. Thus, *in-situ* methods to interrogate biofilm metabolism are needed to fully understand the mechanisms of this disease.

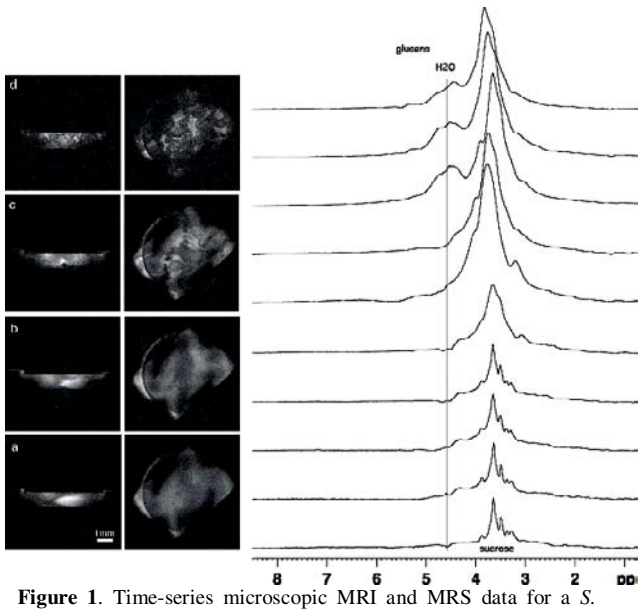

Figure 1. Time-series microscopic MRI and MRS data for a *S. mutans* biofilm attached to a glass coverslip surface and perfused with a sucrose/BHI growth medium. **Left**: Water-selective MRI (a-d; side and top views) of the NMR sample chamber acquired at *t* = 0, 15, 33 and 87 hours after insertion of the ex-situ biofilm. **Right**: Water-suppressed ^{1}H-NMR spectra acquired (from bottom) at *t* = –3, 0, 7, 15, 33, 49, 62, 67, 71 and 86 hours after insertion of the ex-situ biofilm. Broad spectral linewidths are due to the viscous proteinaceous growth medium. The sample temperature was raised from T=25°C to 37°C at *t*=40 hours.

We have recently adapted PNNL's combined confocal scanning laser and nuclear magnetic resonance (CSLM/NMR) microscopy [1] capability for metabolism studies of environmental (*Shewanella oneidensis*) and other biofilms. Bioreactor-grown biofilms on glass microscope-slide surfaces are transferred into the perfused NMR/optical microscope for e*x-situ* observation. CSLM, MRI and localized ^{1}H MRS / MRSI techniques are then used to image biofilm growth and to measure the average [2] and biofilm-depth-resolved [3] concentrations of hydrogen-containing metabolites while maintained in a controlled-growth environment. This study addresses the feasibility of extending MRS measurements to dental biofilms in a model oral environment, thus its utility for elucidating the metabolic mechanisms responsible for tooth decay.

Methods. *Streptococcus mutans* (UA-140 ldh-GFP, kindly provided by Dr. Wenyuan Shi, UCLA School of Dentistry) were pre-grown in 50% brain heart infusion (BHI) media and used to seed glass or hydroxyapatite (HA) surfaces under static or flowing conditions. The samples were transferred to the NMR/CSLM microscope for analysis while perfusing with 10% BHI media containing either sucrose or glucose as substrate.

Results and Discussion. Figure 1 shows time-resolved MRI and localized MRS for *S. mutans* grown on a silica glass surface in sucrose-supplemented 10% BHI medium.

MRI measurements showed rapid growth from an initially thin biofilm, leaving a tortuous flow path after ~30 hours. All MRS data showed significant spectral broadening attributed to a viscous, ill-defined proteinaceous serum composition - a conclusion supported by MRS studies of the filtered media, which yielded similar results. (The line widths obtained were *much broader* than those for the *Shewanella oneidensis* studies employing a chemically defined medium [2,3].) Regardless, a NMR signal increase was observed consistent with carbohydrate resonances, including a spectral signature reported for anomeric glucans [4] (5.2-5.65 ppm range) and qualitatively correlated with the increasing biomass. In another experiment, a *S. mutans* biofilm grown on a HA surface in glucose-supplemented 10% BHI medium yielded slower growth and verified the compatibility of HA for MRI and MRSI studies.

Conclusions. Preliminary results indicate that combined NMR/optical microscopy measurements can be adapted for the study of oral biofilms on relevant growth surfaces. Synthetic enamel and glass biofilm support surfaces are compatible for MRI, MRS and MRSI measurements and the sample chamber developed for *Shewanella* studies is amenable to development of *S. mutans* biofilms, as evidenced by vigorous biomass increase. However, it will be necessary to use thinner enamel surfaces to implement combined NMR/optical measurements. Further, the BHI growth media must be replaced with a chemically- or semi-defined medium to improve NMR spectral resolution. Metabolic studies of biofilms may prove useful for understanding normal and pathological function in dental and other biofilms.

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