The Impact of MEMS-Produced Micro-Electrode Material Coating on Dental Plaque Biofilm Growth

Katherine Warthen
Bioengineering, Montana State University

NNIN REU Site: Lurie Nanofabrication Facility, University of Michigan, Ann Arbor, MI
NNIN REU Principal Investigator: Dr. Carlos Gonzalez-Cabezas, School of Dentistry, University of Michigan
NNIN REU Mentors: Dr. Robert W. Hower, Lurie Nanofabrication Facility, University of Michigan;
Dr. Alexander H. Rickard, Epidemiology, University of Michigan
Contact: kwarthen26@gmail.com, carlosgc@umich.edu, hower@umich.edu, alexhr@umich.edu

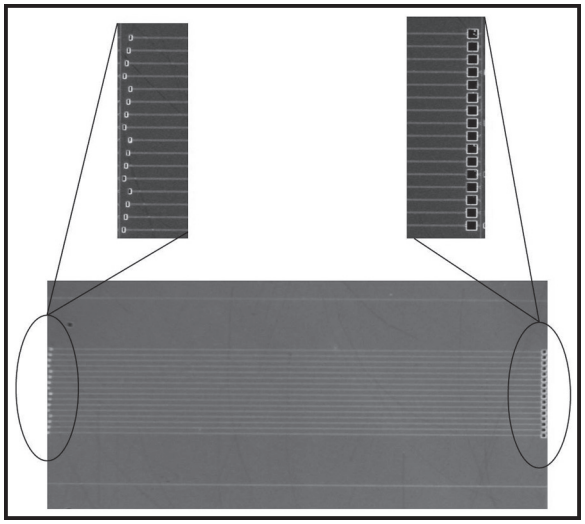

Figure 1: Magnified view of micro pH chip. Close up images are views of the pH sensors (right) and connection terminals for wiring (left).

Abstract:

The development of dental biofilms can create damaging acidic environments due to the production of metabolic byproducts. A micro pH electrode has been created (Figure 1) using microelectromechanical (MEM) fabrication techniques to continuously measure the pH at different distances from saliva-coated surfaces on which biofilms can develop. A coating was needed for this micro pH electrode to prevent the environment of the biofilm and oral cavity from altering the functionality of the pH sensor. Three different material coatings were selected for their known biocompatibility with living cells: parylene, silicon dioxide, and silicon nitride. To determine which coating was best suited for this purpose, the micro electrode was tested in micro flow cells and batch cultures inoculated with fluorescent *Streptococcus gordonii* bacteria. The biofilm was then imaged in three dimensions using a fluorescent microscope.

Medical Relevance:

The oral cavity provides an opportunity for microbes to develop very complex and potentially harmful biofilms. These biofilms can contribute to the formation of dental caries lesions through the production of acidic metabolic byproducts. The longer this biofilm is left undisturbed through substandard dental hygiene, the more likely it is that the caries lesion will worsen [1].

A deeper understanding of the metabolic and acidic conditions in dental biofilms would allow the scientific community to produce better dental hygiene products. Micro-electrode sensors placed within these biofilms could allow for real time data collection of the pH conditions in these micro environments.

Experimental Process:

The micro pH chips were fabricated on silicon wafers. Each chip contained four sets of sensors placed at staggered heights in order to measure pH at different positions within the biofilm. Photolithography techniques such as spinning, evaporation, liftoff, masking, and etching were used to pattern the chips with iridium sensors. The electrochemical sensing sites were then activated by the conversion of iridium to iridium oxide through exposure to an electrically pulsed signal in a sulfuric acid solution (Figure 1).

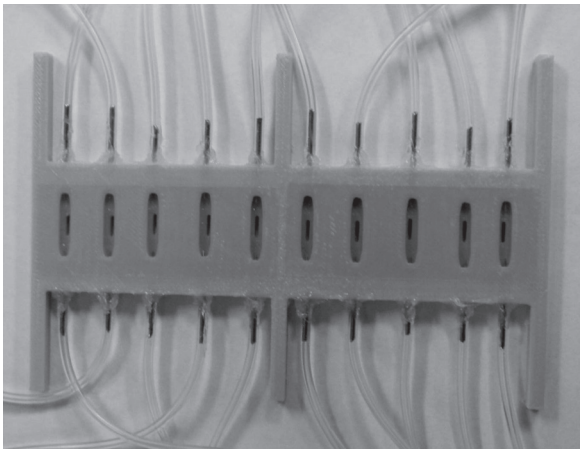

Figure 2: 3-D printed flow cell.

A 3D printer was used to fabricate micro flow cells. A syringe pump modification was also designed to accommodate additional flow channels necessary for efficient testing. The micro flow cells were designed to contain 100 μl of a mixture of human saliva and bacterial growth medium in which the biofilm was cultured (Figure 2).

Discussion:

The micro pH chips produced can successfully signal a change in pH, with an intensity of approximately 20 millivolts per pH unit change (Figure 3). This response can theoretically be improved to between 60 and 80 millivolts per pH unit change following the Nernst Equation and documented iridium oxide electrode responses in the literature [2].

These coatings were then tested in growth media inoculated with *S. gordonii* expressing mcherry, a red protein. Batch experiments conducted on the micro pH chips showed bacterial growth on all materials. Further tests and quantification are needed to determine if there is a significant difference in growth for any material.

Conclusions:

Micro pH sensing chips were produced that can detect a difference in pH conditions in a chemical solution. Three different coatings were tested on these pH micro-electrodes; silicon dioxide, silicon nitride, and parylene. Tests conducted under flow cell conditions showed that *S. gordonii* will grow in the developed flow cell in saliva mixed with growth medium (Figure 4).

Additionally, a new biofilm testing system was developed, consisting of a ten chamber flow cell which could be connected to a syringe pump fitted with a ten syringe expansion attachment. The ten chamber flow cell was custom designed to fit the pH sensing chips into the top of the flow cell. The entire apparatus could then be imaged from below by a confocal microscope to obtain images of biofilm formation on the pH sensing chips in real time (Figure 2).

Future Work:

Future studies will involve improving the accuracy and sensitivity of the sensors, as well as obtaining sufficient biofilm growth for 3D imaging in the flow cell. Eventually the micro pH sensors could be connected to a bluetooth device with an antenna which could be placed into an intraoral retainer to study dental plaque in real time under clinical conditions. The work done during the course of this project serves as preliminary research to produce this type of pH sensor for use in humans.

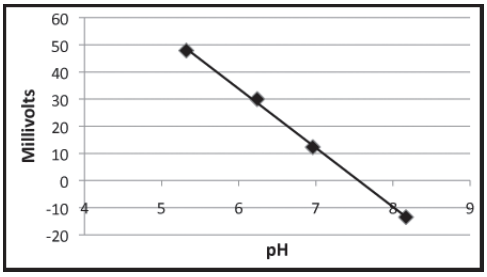

Figure 3: Millivolts emitted by sensor vs. change in pH of solution in which sensor is placed.

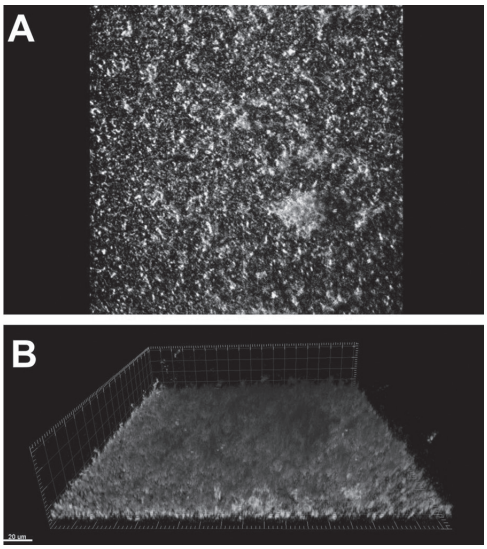

Figure 4: 3-D confocal image of Streptococcus gordonii *biofilm grown in flow cell.*

Acknowledgements:

I would like to thank the labs of Dr. Carlos Gonzalez-Cabezas in the University of Michigan School of Dentistry, Dr. Alexander H. Rickard in the department of Epidemiology, and Dr. Robert W. Hower and Cionca Codrin with the Lurie Nanofabrication Facility at the University of Michigan. In addition, I thank the NNIN REU Program and NSF for funding.

References:

[1] Sachdeo, A., Haffajee, A. D., and Socransky, S. S. Biofilms in the edentulous oral cavity. Journal of Prosthodontics, 5(17), 348-356. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18355168 (2008).

[2] Johnson, M. D., Kao, O. E., and Kipke, D. R. Spatiotemporal ph dynamics following insertion of neural microelectrode arrays. Journal of Neuroscience Methods, 160(2), 276-287. (2006).

[3] Bard, A., and Faulkner, L. Electrochemical methods fundamentals and applications. (2nd ed., V1, pp. 1-83). John Wiley and Sons, Inc. (2001).