

## **Supporting Information**

### **Simultaneous chemical mapping of live biofilm microenvironmental pH and hydrogen peroxide in real-time with a triple SECM tip**

Suji Park<sup>1 †</sup>, Sriram Kumar<sup>1 †</sup>, Claudia S. Maier<sup>1</sup>, Jens Kreth<sup>2\*</sup>, Dipankar Koley<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Oregon State University, Corvallis, Oregon-97331, USA

<sup>2</sup>Department of Restorative Dentistry, Oregon Health & Science University, Portland, Oregon-97201, USA

† Contributed equally

Corresponding author

Jens Kreth - Department of Restorative Dentistry, Oregon Health & Science University, Portland, Oregon-97201, USA; \*Email: [kreth@ohsu.edu](mailto:kreth@ohsu.edu)

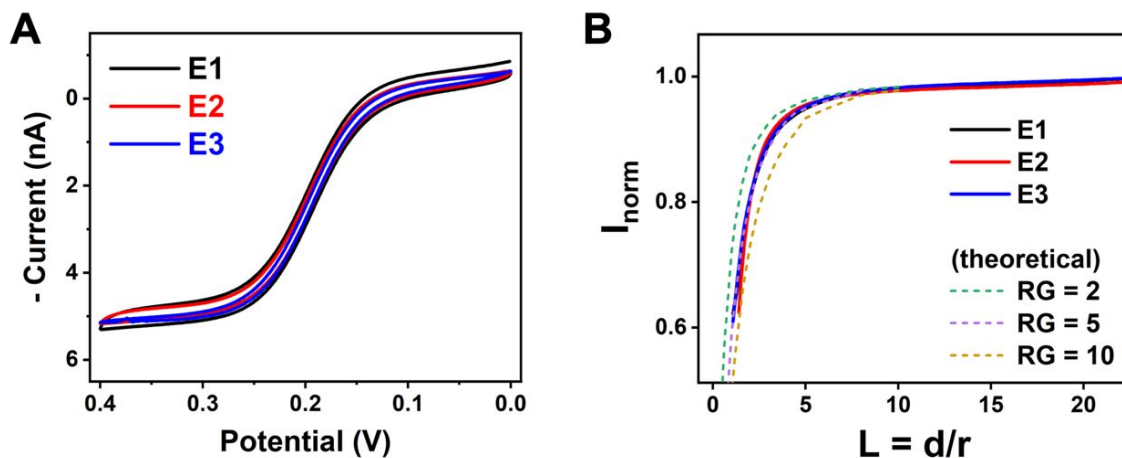
Dipankar Koley - Department of Chemistry, Oregon State University, Corvallis, Oregon-97331, USA; \*Email: [Dipankar.Koley@oregonstate.edu](mailto:Dipankar.Koley@oregonstate.edu)

## Table of content

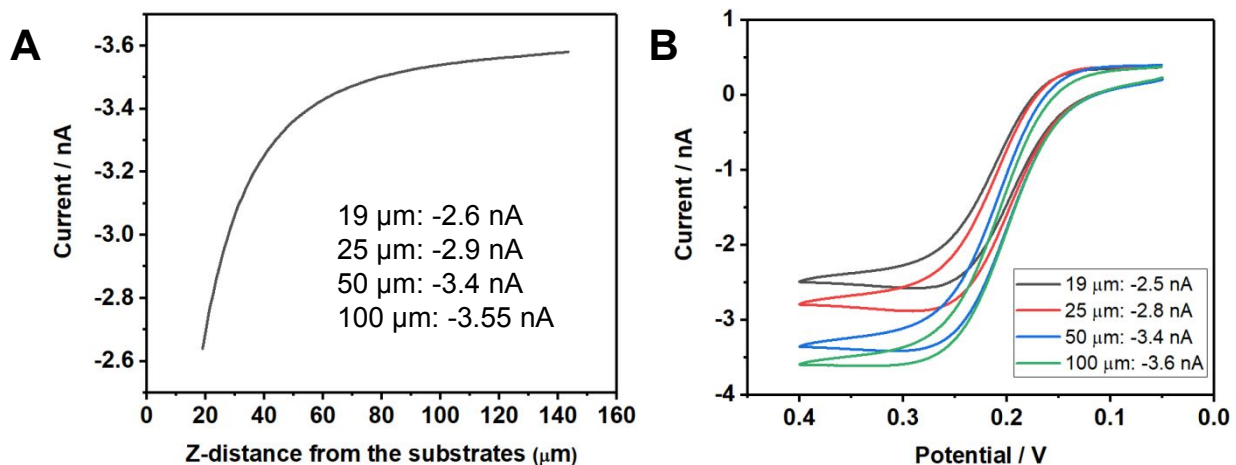
**Figure S1.** Electrochemical characterization of three UMEs in an SECM probe. (A) Cyclic voltammogram of each of the three electrodes at 1 mM ferrocenemethanol in 0.1 M KCl when potential was applied from 0 to 0.4 V vs. Ag/AgCl (1 M KCl) with 0.01 V/s scan rate. (B) Normalized current vs.  $L = d/r$ , where  $r = 12.5 \mu\text{m}$  and  $d =$  distance between probe and substrate, obtained from the probe approach curve in the same solution. .... 3

**Figure S2.** Electrochemical characterization of triple barrel SECM tip. (A) Negative approach curve of SECM tip at insulating substrates in 1mM FcMeOH in 0.1M KCl. The approach curve current at four different theoretical height from substrates ( $19\mu\text{m}$ ,  $25\mu\text{m}$ ,  $50 \mu\text{m}$  and  $100\mu\text{m}$ ). (B) The cyclic voltammetry of SECM tip at four theoretical distances from substrate based on negative approach curve. .... 4

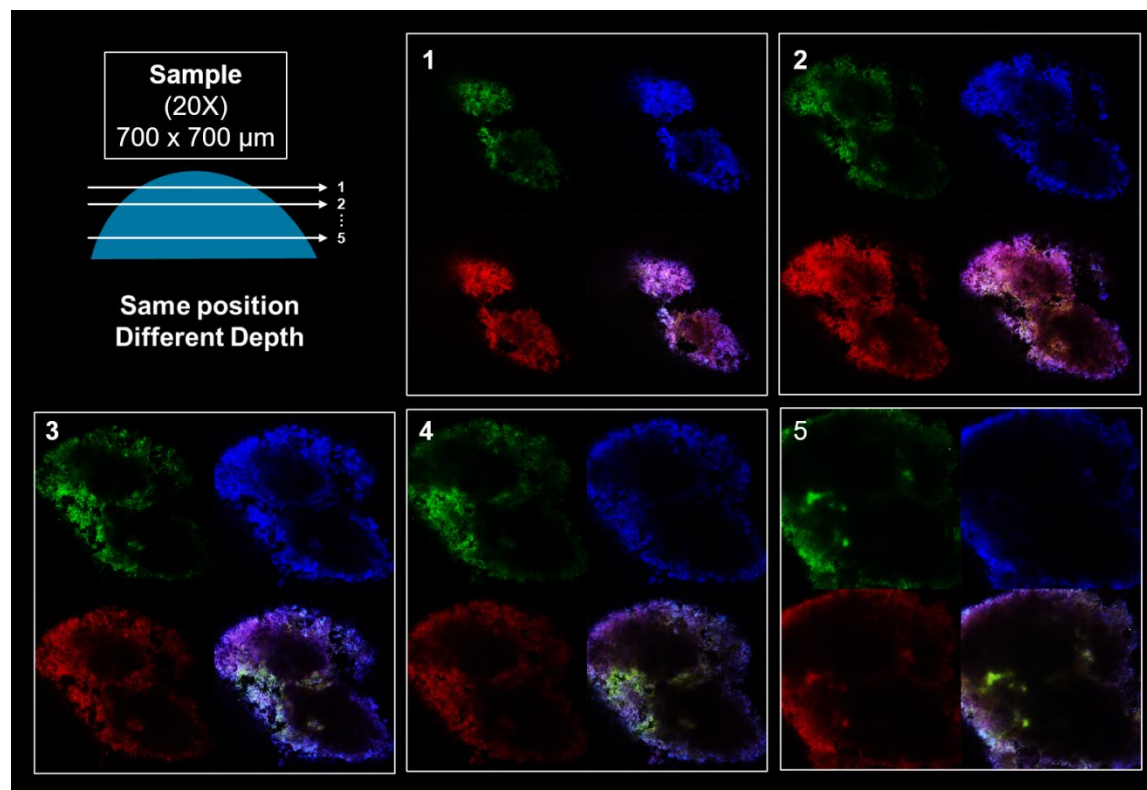
**Figure S3.** Confocal laser scanning microscopy images for visualization of the distribution of bacterial species in live plaque biofilm by using the FISH assay. The images are taken at different depths of the biofilms from 1 (top) to 5 (bottom). In each image slice, the top left represents  $\text{H}_2\text{O}_2$ -producing bacteria labeled with EUB338, the top right represents all bacteria labeled by STR 405, the bottom left represents *Streptococci* bacteria labeled with MIT 447, and the bottom right shows a combination of all three channels..... 5



**Figure S1.** Electrochemical characterization of three UMEs in an SECM probe. (A) Cyclic voltammogram of each of the three electrodes at 1 mM ferrocenemethanol in 0.1 M KCl when potential was applied from 0 to 0.4 V vs. Ag/AgCl (1 M KCl) with 0.01 V/s scan rate. (B) Normalized current vs.  $L = d/r$ , where  $r = 12.5 \mu\text{m}$  and  $d =$  distance between probe and substrate, obtained from the probe approach curve in the same solution.



**Figure S2.** Electrochemical characterization of triple barrel SECM tip. (A) Negative approach curve of SECM tip at insulating substrates in 1mM FcMeOH in 0.1M KCl. The approach curve current at four different theoretical height from substrates (19 $\mu\text{m}$ , 25 $\mu\text{m}$ , 50  $\mu\text{m}$  and 100 $\mu\text{m}$ ). (B) The cyclic voltammetry of SECM tip at four distances from substrate based on negative approach curve.



**Figure S3.** Confocal laser scanning microscopy images for visualization of the distribution of bacterial species in live plaque biofilm by using the FISH assay. The images are taken at different depths of the biofilms from 1 (top) to 5 (bottom). In each image slice, the top left represents H<sub>2</sub>O<sub>2</sub>-producing bacteria labeled with EUB338, the top right represents all bacteria labeled by STR 405, the bottom left represents *Streptococci* bacteria labeled with MIT 447, and the bottom right shows a combination of all three channels.