# Real-time monitoring of cellular oxidative stress during aerosol sampling: a proof of concept study

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Supplemental Information

Electrochemical testing was performed using CV and amperometry. Following Table S1, the hydrogen peroxide concentration of an initial volume of 20 mL buffer solution was systematically increased between 0.01 µM and 10 µM. The solution was stirred after each addition of H2O2 to ensure a uniform concentration throughout the solution. Amperometric testing was performed following Table S2. Unlike CV experiments, which regenerate the reactant by completing the reduction and oxidation of the reactant, amperometric measurements are consuming. This leads to the difference in volumes provided in Table S2.

Table S1. Experimental scheme for calibration curve using CV.

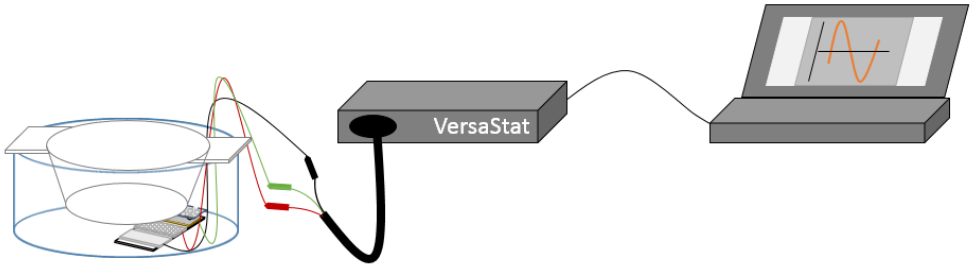
| $H_2O_2$ Concentration (µM) | Volume Added of $H_2O_2$ (mL) | Total Volume (mL) |
|---|---|---|
| 0 | 0.0000 | 20.0000 |
| 0.01 | 0.0020 | 20.0020 |
| 0.05 | 0.0120 | 20.0040 |
| 0.1 | 0.0300 | 20.0160 |
| 0.2 | 0.0602 | 20.0460 |
| 0.4 | 0.1208 | 20.1062 |
| 0.5 | 0.1819 | 20.2270 |
| 0.75 | 0.2547 | 20.4088 |
| 1 | 0.3608 | 20.6636 |
| 3 | 0.8521 | 21.0243 |
| 5 | 1.7705 | 21.8765 |
| 7.5 | 2.9563 | 23.6469 |
| 10 | 4.5980 | 26.6032 |

Table S2. Experimental scheme for amperometric testing of the biosensor.

| Time (s) | $H_2O_2$ Concentration (μM) | Volume Added of $H_2O_2$ (mL) | Total Volume (mL) |
|---|---|---|---|
| 0 | 0 | 0.0000 | 20.0000 |
| 180 | 0.01 | 0.0020 | 20.002 |
| 360 | 0.05 | 0.0100 | 20.012 |
| 540 | 0.1 | 0.0200 | 20.032 |
| 720 | 0.2 | 0.0401 | 20.072 |
| 900 | 0.4 | 0.0803 | 20.152 |
| 1080 | 0.5 | 0.1005 | 20.253 |
| 1260 | 0.75 | 0.1511 | 20.404 |
| 1440 | 1 | 0.2020 | 20.606 |
| 1620 | 3 | 0.6186 | 21.225 |
| 1800 | 5 | 1.0526 | 22.277 |
| 1980 | 7.5 | 1.6216 | 23.899 |
| 2160 | 10 | 2.2222 | 26.121 |

To determine the cellular response in real-time, the sensor was placed on the basolateral side of the cell culture insert within the PIVEC. The electrode leads were then connected to the potentiostat and the change in current was observed on a computer. A schematic of this set-up is observed in Figure S1a. The entire set-up is found in Figure S1b, including the dry dispersal system and PIVEC.

A.




B.

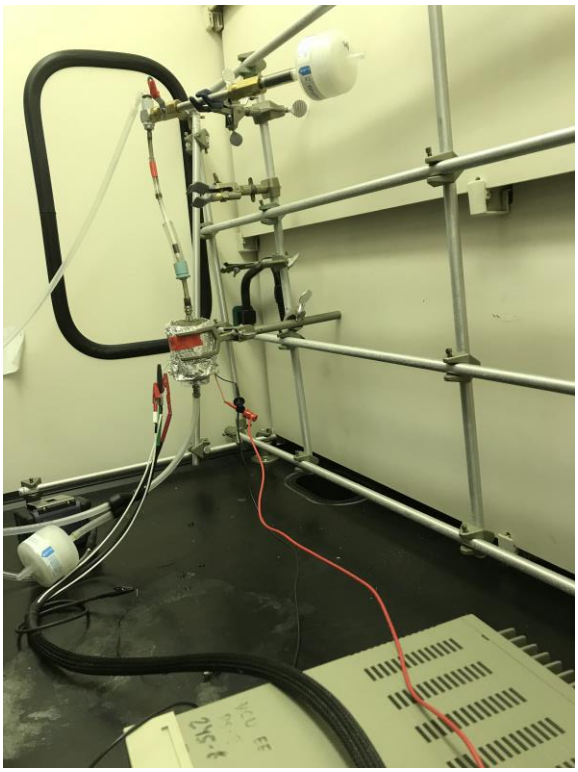
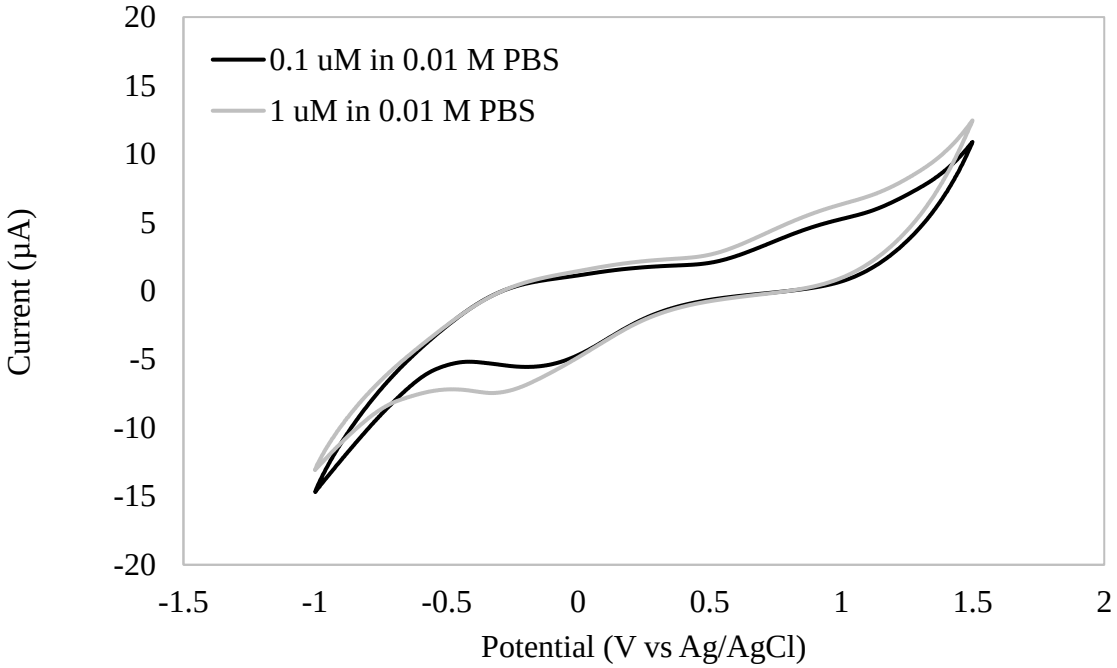


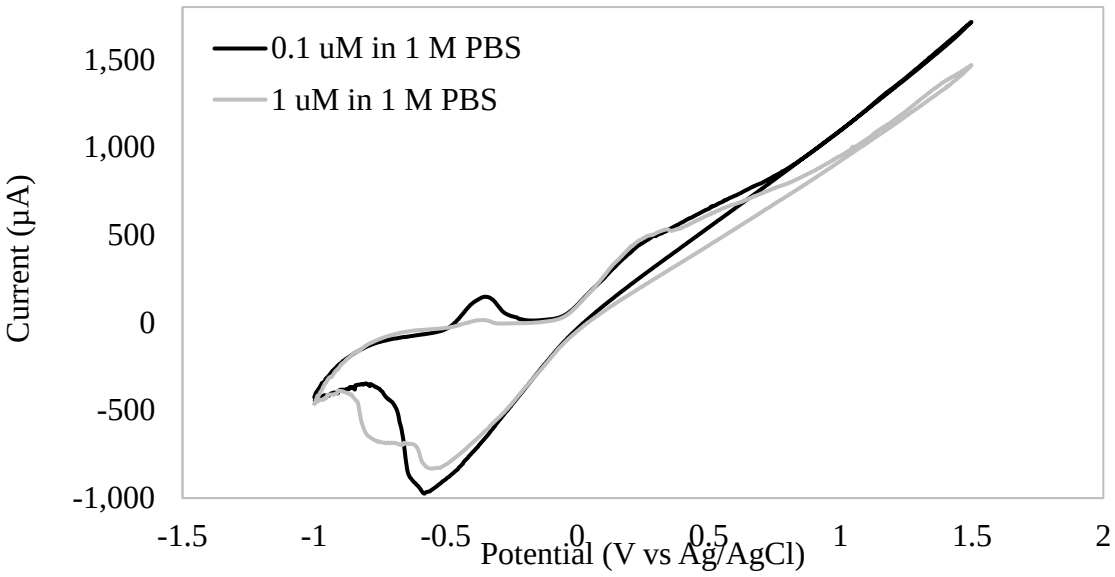
Figure S1. Experimental set-up of real-time ROS generation monitoring. A. Schematic of placement and connection of biosensor. B. Bench top set-up with aerosol generation system and PIVEC.

The sensor was tested in solutions of 0.01 M PBS, 1 M PBS, and HBSS to determine the effects of buffer ionic strength on the sensor response. A low buffer ionic strength, near 1.5 mM, was used and no reaction peaks were observed within the low current measured, Figure S2a. A proportional increase in current was observed when the ionic strength was increased through the

use of 1 M PBS; however no significant reaction peaks were observed, Figure S2b. Through comparing these buffer solutions, Figure S2c, the sensor response within HBSS was the most appropriate for the design used within this study.




A.




B.

C.

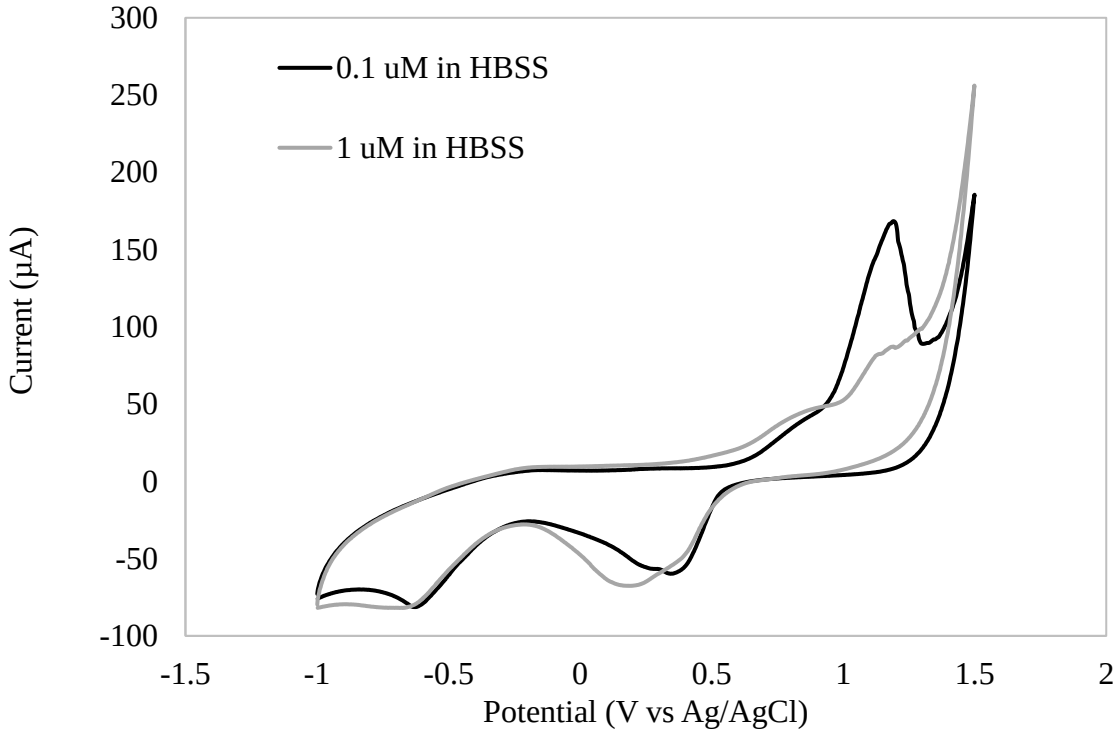



Figure S2. CV response of biosensor to hydrogen peroxide in different buffer solutions. A. Response of 0.1 µM and 1 µM $H_2O_2$ in 0.01 M PBS. B. Response of 0.1 µM and 1 µM $H_2O_2$ in 1 M PBS. C. Response of 0.1 µM and 1 µM $H_2O_2$ in HBSS.