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Cellular Model of Hydrogen Peroxide Release: In Preparation for On-Chip Sensor Measurements

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ABSTRACT

Hydrogen peroxide is traditionally associated with cellular damage; however, recent studies show that low levels of H2O2 are released by cells as part of normal intercellular communication. The mechanisms of hydrogen peroxide transport, uptake and release, and biological effects are not yet well known but have important implications for cancer, stem cells, and aging. Standard H2O2 assays cannot make spatially or temporally resolved quantitative measurements at a cellular scale. Previously we developed a microelectrode array (MEA) and calibration methods for quantifying H2O2 gradients in space and time. The sensor was validated using artificial H2O2 gradients at subsecond and micrometer scale resolutions. The present study begins cellular work on H2O2 release to identify a cellular model system for MEA sensor testing. The morphology and H2O2 release from U937 human monocytes were analyzed after stimulation with ionomycin (1.2 ug/mL) and/or phorbol 12-myristate 13-acetate (PMA). Monocytes were stimulated with PMA (10 ng/mL to 150 ng/mL) for six hours. Hydrogen peroxide release was quantified over time using a traditional amplex red flurometric assay method. Mouse pancreatic beta (MIN6) cells were also tested as a negative control. Monocytes stimulated with PMA alone produced, on average, three times more H2O2 than those stimulated with ionomycin or a combination. Monocytes without ionomycin released H2O2 at 18.34 pmol/min/106 cells at 25 ng/mL of PMA. Ten, 25, and 100 ng/mL of PMA produced H2O2 significantly faster than the non-stimulated control. No significant difference was seen between PMA concentrations when ionomycin was added. These results indicate that PMA stimulated human monocytes may serve as a good model system for cellular validation of the H2O2 MEAs. In the future, biofunctionalization of the electrodes for additional molecular specificity will allow for the expansion of the method to other analytes, giving the sensor potential use in non-traditional lab environments with the ability to perform multiple assays autonomously.

KEYWORDS

Hydrogen peroxide, biosensor, cellular peroxide release, lab on a chip, electrode array, real time sensor