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ABSTRACT

Hydrogen peroxide is traditionally associated with cellular damage; however, recent studies show that low levels of H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> gradients in space and time. The sensor was validated using artificial H<sub>2</sub>O<sub>2</sub> gradients at subsecond and micrometer scale resolutions. The present study begins cellular work on H<sub>2</sub>O<sub>2</sub> release to identify a cellular model system for MEA sensor testing. The morphology and H<sub>2</sub>O<sub>2</sub> release from U937 human monocytes were analyzed after stimulation with ionomycin (1.2 μg/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 fluorometric 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 H<sub>2</sub>O<sub>2</sub> than those stimulated with ionomycin or a combination. Monocytes without ionomycin released H<sub>2</sub>O<sub>2</sub> at 18.34 pmol/min/10<sup>6</sup> cells at 25 ng/mL of PMA. Ten, 25, and 100 ng/mL of PMA produced H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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