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Measurement of hydrogen peroxide influx into cells: Preparation for measurement using on chip microelectrode array

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ABSTRACT

Hydrogen peroxide (H_2O_2) is commonly known as a toxic reactive oxidative species (ROS) for cells. Recent studies have found evidence that H_2O_2 is also an important cellular signalling molecule. Quantifying cellular influx of H_2O_2 will contribute to researchers' understanding of the role H_2O_2 plays in healthy cells and cells involved in the progression of cancers and degenerative diseases. This work utilizes an assay kit and fluorescence techniques to evaluate cell lines and conditions to create a model biological system for measuring cellular H_2O_2 consumption. Pancreatic beta cells (MIN6), astrocytes, and glioblastoma cells (GBM43 and GBAM1) were placed in 10 μM and 20 μM H_2O_2 solutions for up to 5 hours. The consumption of H_2O_2 was measured using an Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes/Invitrogen). GBAM1 cells exposed to 20 μM H_2O_2 displayed the fastest rate of H_2O_2 consumption (4.8 ± 1.2 nmol H_2O_2/min/10^6 cells), followed by GBM43 cells (1.5±0.46), astrocytes (1.1±0.24), and MIN6 cells (0.29±0.075). Additionally, the rate of consumption increased with increases in H_2O_2 concentration. In the future, an on-chip micro-electrode array (MEA) will be used for real-time electrochemical experiments to measure influx of H_2O_2 by astrocytes and GBAM1 cells with spatio-temporal resolution that the current techniques lack. The results from the electrochemical experiments will be compared to results from the assay kit to determine the ability of the MEA to accurately measure H_2O_2 concentration and flux. The MEA can be extended to a wide variety of cellular environments for analysis of additional real-time biological events.

KEYWORDS

Hydrogen peroxide, Biosensors, Microelectrode arrays (MEA), Real-time flux, Astrocytes