Developing cellular model 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 cultured and placed in 10 μM and 20 μM H_2O_2 solutions for up to 5 hours. The consumption of H_2O_2 by these cells over time 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

1. Introduction

H_2O_2 can be produced in cells as a product of aerobic respiration or as a result of the breakdown of O_2^-, a more toxic ROS (Bienert et al. 2006; Lennicke et al. 2015). NADPH oxidases have been found to produce intracellular H_2O_2 in many cells typically as a results of O_2^- elimination (Miller et al. 2010). In addition to production by the cell, hydrogen peroxide can also enter the cell from the external environment. When entering cells, H_2O_2 has limited membrane permeability, so it can partially diffuse across the membrane and also relies on transport through aquaporins, especially through Aquaporin 3 and Aquaporin 8 (Lennicke et al. 2015; Miller et al. 2010; Watanabe et al. 2016). Once inside the cell, hydrogen peroxide plays a variety of roles. A newly recognized benefit of hydrogen peroxide is its ability to modify different proteins, such as cysteine residues, by oxidizing them and preparing them for different actions throughout the cell, such as DNA transcription (Lennicke et al. 2015). However, a surplus of H_2O_2

in the cell can cause oxidative stress, which can have damaging effects on the cell: The high levels of H_2O_2 trigger pathways that can result in membrane or DNA damage or apoptosis (Bienert et al. 2006; Lennicke et al. 2015). To regulate the levels of H_2O_2 in cells, there are different enzymes to help breakdown H_2O_2 into less dangerous molecules. The two main enzymes used to eliminate H_2O_2 and break it down into H_2O and O_2 are catalase and glutathione peroxidase (GPx). In addition, there are other systems, including peroxiredoxins, to eliminate smaller amounts of hydrogen peroxide (Hashida et al. 2002; Lennicke et al. 2015; Sasaki et al. 1998).

Different cells have different rates of H_2O_2 removal depending on the amounts of catalase, GPx, and other anti-oxidant enzymes present (Makino et al. 2008; Wagner et al. 2013). In addition to variations between different healthy cells, cancer cells also have different removal rates from healthy cells. The increased rate of hydrogen peroxide removal by cancer cells can give insight into their methods of survival in the body and possible treatment methods centered on the elimination and use of H_2O_2 in cancer cells (Lennicke et al. 2015).

There are a variety of methods that have been used to measure cellular removal rates of hydrogen peroxide. In order to measure both intra and extracellular amounts of H_2O_2, many groups use chemiluminescence (Hashida et al. 2002; Makino et al. 1994) or fluorescence techniques using different assay kits (Amatore et al. 2015; Wagner et al. 2013; Yamada et al. 2000). Once the amount of hydrogen peroxide is measured, some groups then use enzyme kinetics of catalase and GPx to create a mathematical model quantifying the rates of removal (Hashida et al. 2002; Makino et al. 2008; Makino et al. 1994; Sasaki et al. 1998; Wagner et al. 2013).

A newer method for detecting extracellular H_2O_2 is the use of electrochemistry. With this method, electrodes are coated in a material, such as platinum or platinum black, which undergoes a redox reaction with H_2O_2 (Chang et al. 2013; Li et al. 2013; McLamore et al. 2011). The redox reaction between hydrogen peroxide and metal causes the oxidation of hydrogen peroxide. The two electrons that are lost by the H_2O_2 create a current that can be measured. Additional calibration of the electrode sensors is used to relate the measured current to the concentration, flux, and removal rate of hydrogen peroxide in the environment around cells (Amatore et al. 2015; Chang et al. 2013; Li et al. 2013; Madangopal et al. 2012; McLamore et al. 2011; Rothe et al. 2012; Shi et al. 2011). Self-referencing probes are a common method to apply this electrode approach. In self-referencing, an electrode is moved between two points to detect the local concentration gradient close to and farther from the cell (McLamore et al. 2011; Shi et al. 2011). In our group, extensive work has been done with the self-referencing technique, and now we are developing microelectrode arrays (MEAs) to detect concentration gradients around cells without the need to move electrodes around cells. This new method can allow for multiple, real-time measurements to be taken.

This study uses a traditional assay kit and fluorescence techniques to investigate a variety of cell types to determine what cell line and conditions are able to create a model biological system for detecting cellular H_2O_2 consumption. Some cells, such as astrocytes, are known to consume H_2O_2; however, for other cell types, like pancreatic beta cells and glioblastoma cells, the measurement of H_2O_2 consumption is more novel. In the future, the cell lines and conditions in the model system will be used to investigate the efficacy of a chip that is fabricated with a MEA to measure the flux of H_2O_2 into different types of cells under physiologically relevant conditions. The ability to place the array of micron-scale electrodes on a chip platform will create the ability to capture real-time measurements of biological events with increased spatial and temporal resolution that the current optical techniques lack. In addition, the on-chip MEA will increase portability of the device and will also allow for measurements to be taken in a wide variety of environments with small number or large groups of cells.

2. Materials and Methods

2.1 Cell Culture

MIN6 (mouse pancreatic beta cells) cells were cultured in DMEM (high glucose), which was supplemented with 15% FBS (fetal bovine serum) (Life Technologies). To create pseudo-islets for testing, cells were placed into an ultra-low attachment 6 well plate (Corning) with 3 mL media and allowed to aggregate for four days prior to testing.

Human astrocytes (ScienCell) were cultured in flasks coated with poly-L-lysine with Astrocyte Medium composed of basal medium (ScienCell) supplemented with 2% FBS, 1% penicillin/streptomycin solution (P/S), and 1% astrocyte growth supplement.

Human-derived glioblastoma cells (GBM43) were provided by Dr. Karen Pollok and Dr. Aaron Cohen at Indiana University and the Methodist Hospital of Indianapolis. GBM43 cells were cultured in poly-L-lysine coated flasks with DMEM (high glucose) and supplemented with 10% FBS (Life Technologies).

Stem-like glioblastoma cells (GBAM1) were provided by Dr. Phillip Tofilon and the Moffitt Cancer Center. GBAM1 cells were cultured in poly-L-lysine coated flasks with DMEM/F12 supplemented with B27 (Invitrogen).

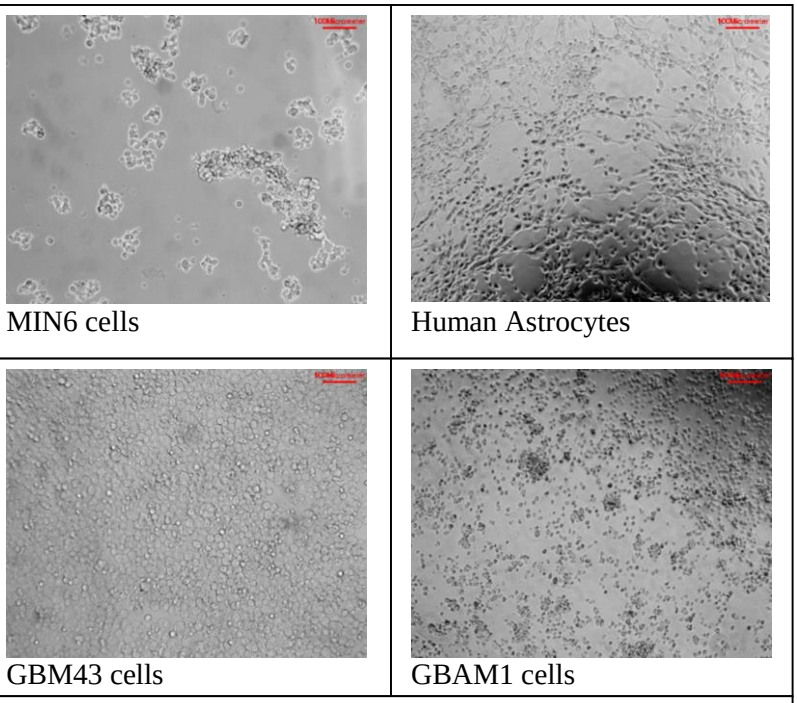


Fig 1. Images of all cell lines used. All images taken with light microscope camera, 10 x. Scale bar = 100 micrometers

Human astrocytes, GBM43, and GBAM1 cells were placed in poly-L-lysine coated wells of a 96 well plate 18 hours before testing started to allow the cells time to adhere to the well plate and develop.

All cell lines (seen in Fig. 1) were incubated at 37 ˚C between passages. In addition, the media was changed every other day and cells were passaged each time they reached 90% confluence.

When setting up the cellular experiments, the cells in flasks were covered in 0.025% Trypsin EDTA and incubated for 3 to 5 minutes. Then cells were removed from the flasks and put into a centrifuge to separate the cells from the liquid. The cells were re-suspended in buffer or media and then counted using Trypan blue staining and a Countess Automatic Cell Counter (Invitrogen) to determine cell densities for each experiment. After counting the cells, they were pipetted into 96 well plates to be used for experimentation with the assay kit. Astrocytes, GBAM1, and GBM43 cells were adherent to the bottoms of the 96 well plates due to the poly-L-lysine coating, while the MIN6 cells remained suspended throughout the experiment.

2.2 Assay Kit and Fluorescence Techniques

2.2.1 Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit

An Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes/Invitrogen) was used to obtain initial measurements of hydrogen peroxide consumption. The Amplex Red reagent mixture was created using the kit protocol and was added to a different mixture of buffer, media, cells, and H_2O_2

depending on the experiment. The Amplex Red Reagent is combined with horseradish peroxide, and in the presence of H_2O_2, Amplex Red is oxidized into resorufin, a red fluorescent molecule. The reaction was carried out in clear, flat-bottom 96 well plates that contained the different cells and solutions and was incubated for 30 minutes at 37 ˚C. Fluorescence (excitation 545 nm, emission 590 nm) and absorbance (560 nm) measurements, were taken with a BioTek Synergy NeoB plate reader after the 30-minute incubation period.

For each assay plate created, there was a standard curve made to calibrate the assay and to convert from raw fluorescence values to H_2O_2 concentrations. The final standard curve was created using serial dilution of an original H_2O_2 solution in which 150 µL of solution from the previous well was added to 50 µL of buffer to obtain a new concentration that was 75% of the previous concentration. The final standard calibration curve had values of 0, 1.1, 1.5, 2, 1.7, 3.6, 4.6, 6.3, 8.4, 11.3, 15 and 20 µM. Triplicates of the standard curve and each condition tested were used to ensure better reliability of the results. The fluorescence and absorbance measurements from each of the three triplicates was averaged to obtain final H_2O_2 concentrations after each time period. The results from wells that included cells were compared to the standard curve at the same time point to ensure changes in concentration was due to cellular consumption instead of developing of the Amplex Red reagent.

2.2.2 Initial Experiment

For the initial experiments, pseudo-islets of MIN6 cells were cultured for 4 days in ultra-low attachment 6 well plates (Corning) with approximately 500,000 cells per well. The majority of the media was removed from each well and replaced with 3 mL of a buffer and H_2O_2 solution. Final concentrations of hydrogen peroxide were 10 µM, 20 µM, and 40 µM. Every 5 minutes for 25 minutes, 50 µL of the buffer surrounding the cells was removed and placed into a 96 well plate for use with the assay reagents. Each time point was tested in triplicate. After all time points were collected, 50 µL of the Amplex Red reagent mixture was added to each well (see Fig. 2A). The kit was incubated at 37 ˚C for 30 minutes, and fluorescence and absorbance measurements were taken according to the specifications above.

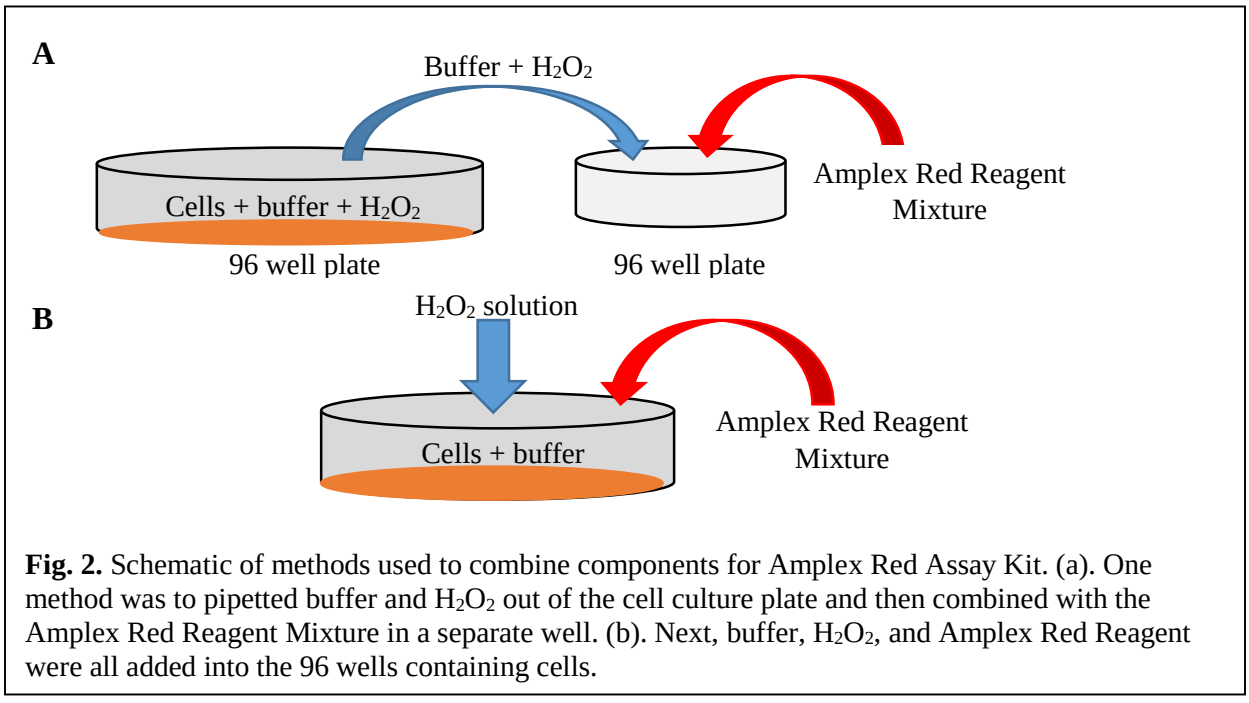


Fig. 2. Schematic of methods used to combine components for Amplex Red Assay Kit. (a). One method was to pipetted buffer and H_2O_2 out of the cell culture plate and then combined with the Amplex Red Reagent Mixture in a separate well. (b). Next, buffer, H_2O_2, and Amplex Red Reagent were all added into the 96 wells containing cells.

2.2.3 Secondary Experiment

In a subsequent experiment, astrocytes, MIN6 cells, and GBAM1 cells were suspended in buffer and 25 µL of the cell mixture was placed directly into a 96 well plate. Astrocytes were seeded with approximately 50,000 cells per well, MIN6 cells had approximately 62,000 cells per well, and GBAM1 cells had approximately 10,000 cells per well. At different time points, 25 µL of 20 µM or 40 µM H_2O_2 solution was added to the wells so that, after mixing, cells were exposed to final H_2O_2 concentrations of 10 µM and 20 µM. GBAM1 cells were only exposed to 20 µM H_2O_2. Cells were exposed to H_2O_2 for 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 minutes. Again, each condition was tested in triplicate to improve the reliability of the results. After the final time point was completed, 50 µL of Amplex Red reagent mixture was added to all wells to measure the amounts of extracellular H_2O_2 in the wells (see Fig. 2B). Fluorescence and absorbance measurements were taken with the plate reader 30 minutes after the addition of Amplex Red reagent.

2.2.4 Final Assay Kit Experiment

Final experiments used the initial method of removing the solution surrounding cells and mixing this in a second well with the Amplex Red Reagent (Figure 2A). In this experiment, astrocytes and GBM43 cells were seeded with approximately 50,000 cells per well in a poly-L-lysine coated flat bottom, 96 well plate. After sitting overnight, media was removed from the wells and replaced with 200 µL of 20 µM H_2O_2. At different time points (5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes), 50 µL of solution from the wells were removed at placed in a second clear 96 well plate. After all time points were completed, 50 µL of Amplex Red Reagent was added to all wells. The plate was incubated for 30 minutes and fluorescence measurements were taken.

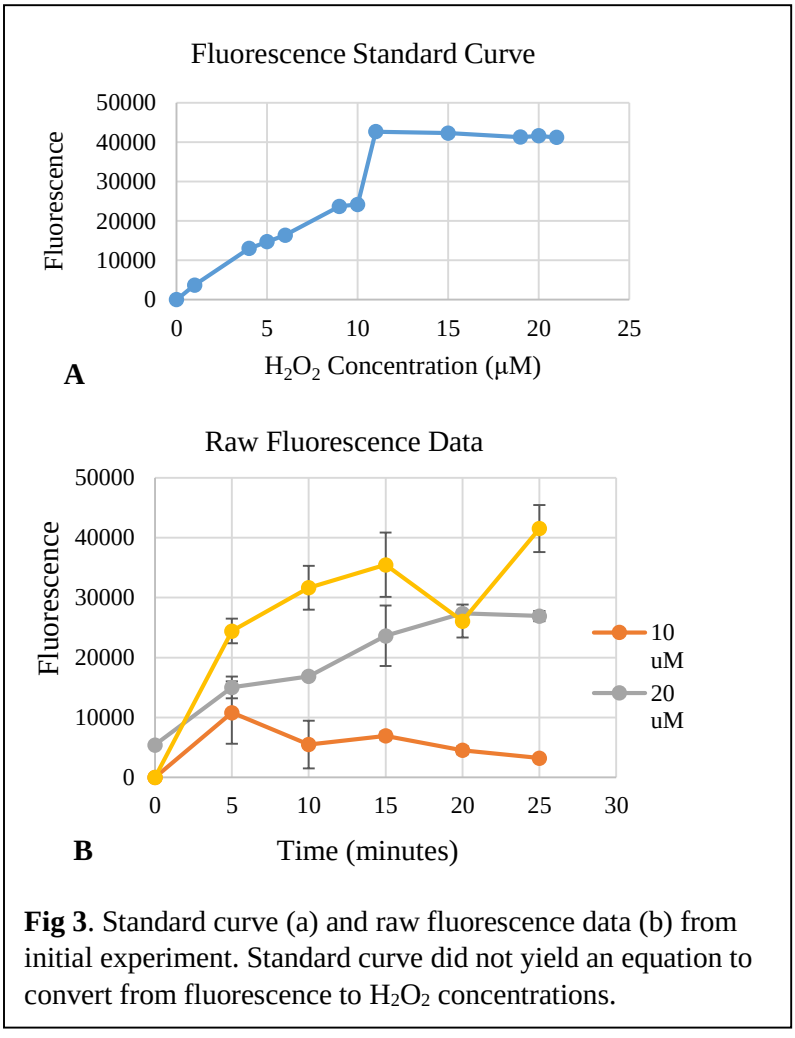


Fig 3. Standard curve (a) and raw fluorescence data (b) from initial experiment. Standard curve did not yield an equation to convert from fluorescence to H_2O_2 concentrations.

3. Results

3.1 Initial Experiment Results

The standard curve made in the initial experiment did not use serial dilution and was not completed in triplicate, so it did not yield an accurate curve with an equation that could be used to calculate H_2O_2 concentration from the raw fluorescence data recorded (Fig. 3). However, fluorescence values showed an increase over the 25-minute time period, which might have indicated an increase in H_2O_2 concentration over time, and provided justification for further testing.

3.2 Secondary Experiment Results

In the second protocol used, the serial dilution described in section 2.2.1 was used in triplicate and yielded a linear line with the equation y = 4497.9x + 2632.6 and an R^2 value of 0.9925 (Figure 4). This equation was corrected to account for a discrepancy between wells with only buffer and no H_2O_2 and wells with cells, buffer, and no H_2O_2. To do this, the well with cells, buffer and no H_2O_2 was subtracted from all values in the standard curve.

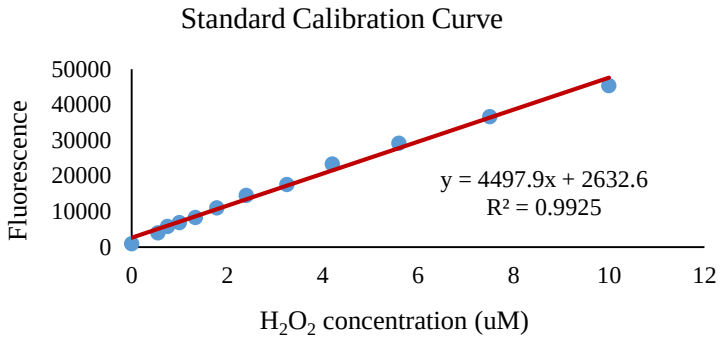


Fig. 4. When using the assay kit, a standard curve of different H_2O_2 concentrations was created in triplicate using serial dilution and was used to convert measured fluorescence values into concentrations of H_2O_2. This is the standard curve for the secondary experiment protocol.

Astrocytes, MIN6 cells, and GBAM1 cells consumed H_2O_2 at rates that fit an exponential decay function (Figure 5). The rates of H_2O_2 consumption was determined using the slope from the ln(H_2O_2) vs Time graph using the method described by Wagner et al. (2013) (Figure 5). The observed slope had units of 1/min. The slope was divided by the cell density for each cell type to obtain a rate constant, k, with units of L min^{-1} $cell^{-1}$. The rate constant was then multiplied by the concentration tested (10 or 20 µM) and divided by 10^6 to obtain a consumption rate in units of nmol H_2O_2 / min / 10^6 cells. This normalized rates were then able to be used to compare the consumption rate of H_2O_2 measured from each condition tested (Table 1).

GBAM1 cells reached a 0 µM H_2O_2 solution between 1 and 1.5 hours. Astrocytes were able to consume H_2O_2 and achieve a 0 µM solution in approximately 2 hours. MIN6 cells also consumed H_2O_2 down to a 0 µM solution; however, consumption by the beta cells took approximately 4 hours.

For astrocytes and MIN6 cells, the consumption rate for cells exposed to 20 µM H_2O_2 was approximately 2 times greater than the consumption rate for the same cells exposed to 10 µM H_2O_2.

3.3 Final Assay Kit Experiment Results

Similar to the results from experiment 2, the astrocytes and GBM43 cells exposed to 20 µM H_2O_2 had Concentration vs. Time curves that displayed exponential decay (Figure 6). The rates of consumption were calculated using the rate constant, k, given by the slope of the ln(H2O2) vs. time graph and the method used by Wagner et al that is described in section 3.2.

The astrocyte consumption rate (0.8 ± 0.045 nmol H_2O_2 / min / 10^6 cells) was slightly lower than the rate from experiment 2. This discrepancy might be due to the variation in protocol between leaving the cells in the well versus pipetting out some of the solution. From this experiment, the GBM43 cells displayed a faster consumption rate than the human astrocytes (Table 1).

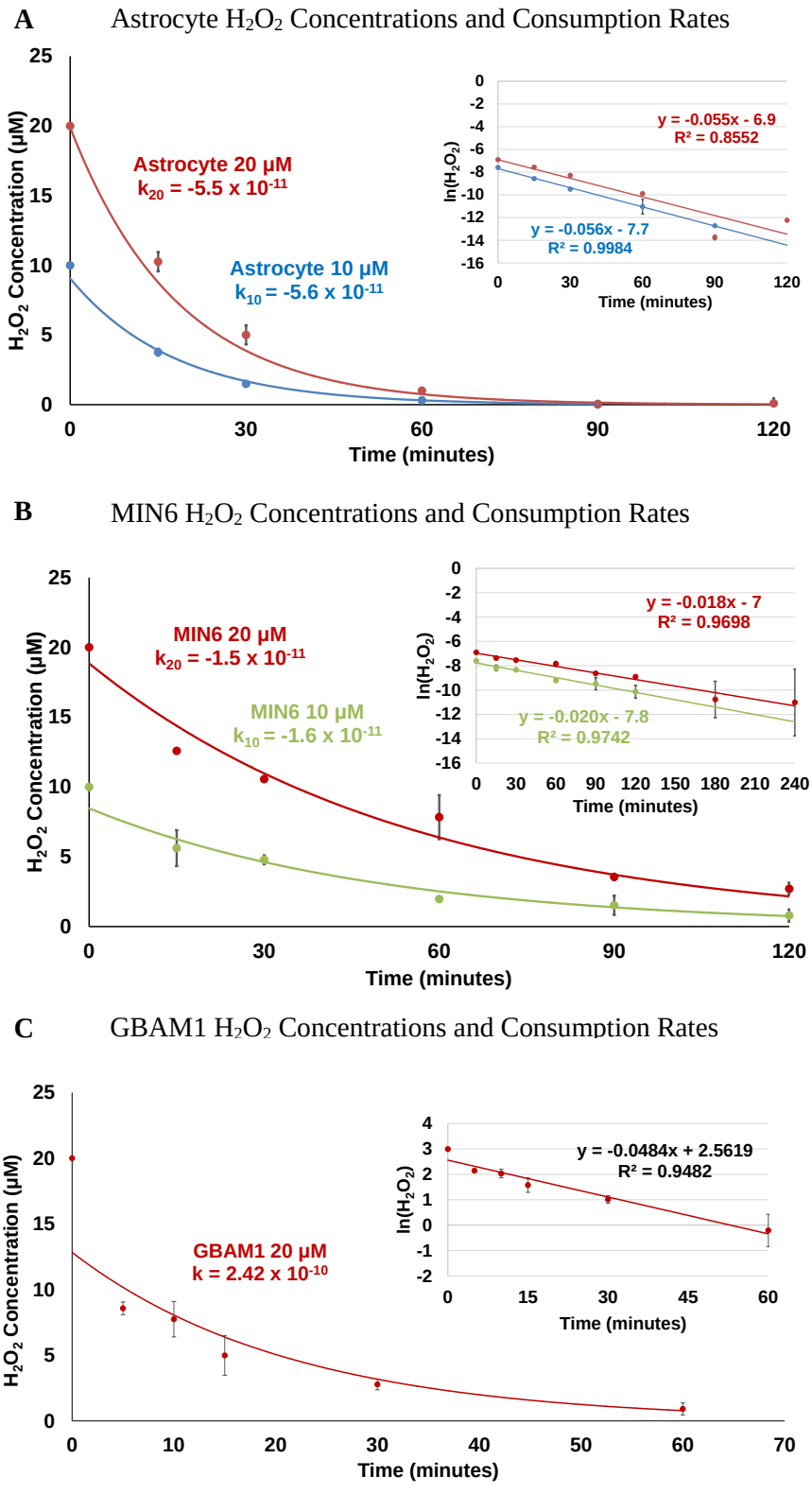
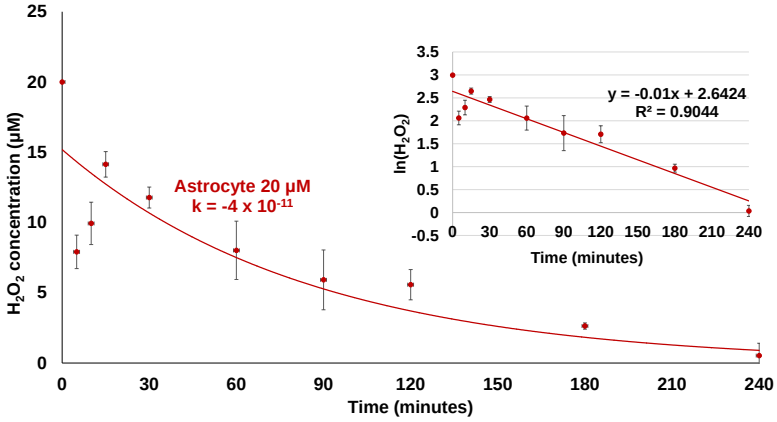


Fig. 5 Concentration and consumption rates from secondary experiment with MIN6 cells (a), astrocytes (b) and GBAM1 cells (c). GBAM1 cells displayed the fastest rate of consumption, followed by astrocytes and then MIN6 cells. The 20 μM H_2O_2 solution promoted faster consumption rates than the 10 μM H_2O_2.

Table 1. Rate constants and consumption rates for all cell lines exposed to 20 μM H_2O_2. The rate constant, k, was multiplied by the concentration tested and by 10^6 to normalize rates to the specified units to allow for comparison between different conditions. GBAM1 cells displayed the fastest rate of H_2O_2 consumption over time. The negative k indicates consumption of H_2O_2 instead of production.

Cell line	Rate constant, k ($L\ min^{-1}\ cell^{-1}$)	consumption rate (nmol H_2O_2 / min / 10^6 cells)
GBAM1	-2.4×10^{-10}	4.8 ± 1.2
GBM43	-7.5×10^{-11}	1.5 ± 0.46
Human Astrocytes	-5.5×10^{-11}	1.1 ± 0.24
Pancreatic Beta Cell (MIN6)	-1.5×10^{-11}	0.29 ± 0.075

A Astrocyte 20 μM H_2O_2 Concentrations and Consumption Rates



B GBM43 20 μM H_2O_2 Concentrations and Consumption Rates

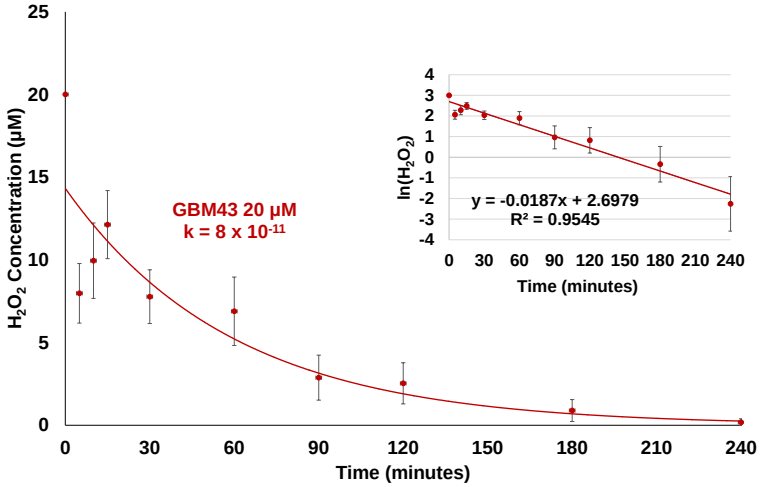


Fig. 6. Results from Experiment 3 with GBM43 cells and astrocytes in 20 μM H_2O_2. GBM43 cells consumed H_2O_2 faster than astrocytes.

4. Discussion

4.1 Differences between cell lines

The two main categories of cells tested where pancreatic beta cells (MIN6) and astrocyte-like cells (human astrocytes, GBM43, and GBAM1). Of these two types, the cells that were astrocyte-like displayed much faster rates of H_2O_2 consumption than the beta cells. These results are consistent with the known roles of each cell type.

Astrocytes are a type of glial cell in the central nervous system that protect and support neurons. Since the brain is a primary user of oxygen due to the high metabolic level of cells in the brain, the environment is very susceptible to oxidative stress (Desagher et al. 1996; Milton 2004). In order to minimize any damages from oxidative stress, astrocytes are specialized to consume H_2O_2 and other free radicals in order to break them down into less toxic molecules. In order to consume large amounts of H_2O_2, astrocytes have high levels of proteins like catalase and GPx that break down the consumed H_2O_2 (Makino et al. 2008). On the other hand, beta cells, like MIN6, are known to have low levels of GPx, which hinders their ability to convert consumed H_2O_2 into water and oxygen (Robertson and Harmon 2007). Due to their protein deficiency, it is understandable that the MIN6 cells would have slower consumption rates than the high protein-containing astrocytes. However, despite their lack of proteins, the MIN6 cells were still able to consume H_2O_2 down to a concentration of 0 μM. The ability of the MIN6 cells to consume all of the H_2O_2 present indicates that cells besides those that are specific for H_2O_2 consumption have the capacity to eliminate H_2O_2 in order to create a more favorable environment for the cells to grow in.

The glioblastoma cells (GBM43 and GBAM1) are cancerous cells that originate from astrocytes. Since they are astrocyte derivatives, it can be hypothesized that these cells possess similarly high levels of H_2O_2 consuming proteins like catalase and GPx. In addition, cancer cells are known to have high levels of H_2O_2 dependent signaling pathways associated with cell growth and survival and high levels of ROS as a result of the increased metabolism of cancerous cells (Lennicke et al. 2015). Increased H_2O_2 signaling and increased levels of ROS require high levels of proteins to breakdown H_2O_2 and other ROS to allow the cancerous cells to continue to thrive. Both GBM43 and GBAM1 cells displayed faster H_2O_2 consumption rates than the normal human astrocytes. This confirms that ability of cancer cells, and glioblastoma cells specifically, to survive in environments that are high in hydrogen peroxide. Since high levels of H_2O_2 can be toxic to healthy cells, the ability of cancer cells to consume and breakdown H_2O_2 quickly gives them an edge in surviving in the body.

4.2 H_2O_2 consumption is concentration dependent

In the experiment described in section 2.2.3, consumption rates were measured for astrocytes and MIN6 cells exposed to 10 and 20 μM H_2O_2 solutions. These two concentrations were chosen for measurement because they both fall within the typical range of H_2O_2 exposure for different types of cells and both concentrations are minimally damaging to cells (Halliwell et al. 2000). In initial experiments, MIN6 cells were exposed to 40 μM H_2O_2; however, after exposure cells were dark which indicated stress due to the H_2O_2 levels. Additionally, measuring H_2O_2 levels greater than 20 μM reduces the accuracy of the assay kit used, so using concentrations equal to or below 20 μM would be able to optimize the use of the assay kit.

For both cell lines, the consumption rate for cells exposed to 20 μM H_2O_2 was approximately double the rate of cells exposed to 10 μM H_2O_2. These results indicate that the cellular consumption of H_2O_2 in this range is concentration dependent and not reaction dependent.

When moving forward with electrochemical sensing with the MEA, it will be most beneficial to measure cellular consumption rates of cells exposed to high levels of H_2O_2 in order to ensure that the consumption rates are fast enough to test the ability of the electrodes to accurately measure the real-time consumption of H_2O_2. Since the MEA will not be limited to a maximum concentration of H_2O_2 it can

detect like the assay kit is, it will be possible to test cells exposed to higher than 20 μM H_2O_2 in order to further study the ranges in which cells are able to consume H_2O_2 without significant damage and it will be possible to see if the concentration dependence holds true for higher concentration ranges.

4.3 Influx of H_2O_2

To further understand the consumption of H_2O_2 by the 4 different cell types, the flux of H_2O_2 into the cells was calculated in units of picomoles / cm^2 / sec. To make this calculation, rate of consumptions (given in Table 1) were divided by the surface area of one cell and conversions were made to obtain the specified units. Table 2 shows the cells areas used and the approximate calculated flux values. Since the sizes are approximated from previous data (Milo et al. 2010) and was not measured for each cell line individually, there may be a wide flux range to consider and more accurate measurements would need to be taken to verify these amounts.

Table 2. Influx rates of 4 different cell lines used. To calculate flux, consumption rates from Table 1 were divided by the cell surface area, which was approximated using the given cell diameters and the equation for the surface area of a flat circle ($SA = \pi r^2$). Cell sizes were approximated using previously measured values for the different cell types (Milo et al. 2010).

Cell line	Cell diameter (μm)	Flux (pmol / cm^2 / sec)
GBAM1	10	900
Human Astrocytes	8	40
GBM43	12	20
Pancreatic Beta Cell (MIN6)	10	6

The electrochemical sensors that will be used to measure influx of H2O2 by these different cells are accurate to approximately 30 pmol / cm^2 / sec. In order to create the most optimal biological system to test the MEA on a chip, GBAM1 cells and astrocytes should be used first because they have fluxes within the range of the current electrode sensors. As the sensors become more accurate, GBM43 cells offer another good model system to test the efficacy of the MEA.

5. Conclusion

Astrocyte-like cells displayed the fastest H_2O_2 consumption rates and influx values, and thus provide an optimal biological model that can be used to measure cellular H_2O_2 consumption. The stem cell-like glioblastoma cells (GBAM1) consumed H_2O_2 faster than normal astrocytes and the other glioblastoma cells lines, which makes them the ideal biological model for real-time measurements with a MEA. The high consumption rates of the GBM43 and GBAM1 cells also validate that cancer cells do have increased abilities to combat high H_2O_2 levels and avoid oxidative stress, which contributes to their risk. In addition to their rapid consumption of H_2O_2, astrocytes, GBM43 and GBAM1 cells are an ideal cellular model to be used in sensor testing because they are adherent to the bottom of well plates, which will allow for a sensor to be brought a known distance away from the cells to allow for increased spatial resolution of flux measurements.

When creating the biological system for measure cellular consumption of H_2O_2, it was found that up to 20 μM H_2O_2, consumption is concentration dependent, so testing cell exposed to higher concentrations of H_2O_2 should provide the largest measureable flux values. Future electrochemical experiments will be able to further validate this and test a wider range of concentrations to determine how large of a range this relationship holds true for.

6. Future Work

In future work, a MEA will be used to measure the influx of hydrogen peroxide into the astrocytes and glioblastoma cells exposed to 20 μM H_2O_2 and other H_2O_2 concentrations. The proposed MEA will have an array of 10 platinum electrodes fabricated in a nanofabrication clean room and placed on a silicon chip (Fig. 7). The MEA will be used to conduct electrochemical experiments to detect, in real time, the flux of hydrogen peroxide into astrocytes and glioblastoma cells as they are exposed to a variety of H_2O_2 concentrations. Each electrode in the MEA will measure the change in a local concentration of H_2O_2 over time and the combined results from the different electrodes in the array will be used to calculate influx of hydrogen peroxide. The results from future electrochemistry experiments using the MEA will be compared with the concentration results from the Amplex Red assay kit experiments used here to determine the accuracy of the MEA in this concentration range. The MEA will also be used to measure cellular consumption in a wider concentration range to determine the concentrations for which consumption of H_2O_2 is concentration dependent. The MEA can be further modified with different polymer coatings to sense for glucose, lactate, and other biological molecules.

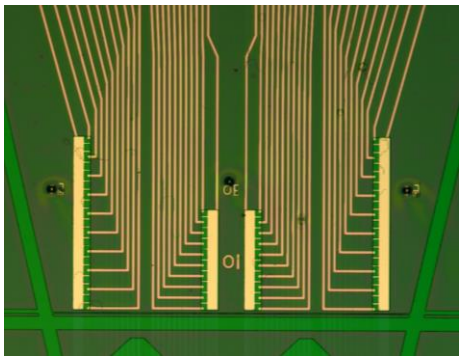


Fig. 7. 4 fabricated MEAs each with ten 10 μm x 10 μm electrodes. This array will be placed into a solution with cells adherent to the bottom of the container in order to conduct electrochemical measurements of H_2O_2 influx with spatio-temporal resolution that the assay technique lacks.

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