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Modeling of an Oxygenation-Aided 3D Culture for Functional Beta-Cell Expansion

An Undergraduate Honors College Thesis

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By

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This thesis is approved.

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Summary

All over the world the prevalence of the debilitating disease, diabetes, is increasing rapidly. Diabetes is characterized by the inability to regulate blood-glucose levels. A new treatment for diabetes, with much potential, is the transplant of insulin-producing beta cells into diabetic patients in order to increase a patient’s beta cell mass. The main issue with pancreatic tissue transplants is the lack of available donor tissue. A potential source of tissue for transplantation is the proliferation of beta cells in vitro. Beta cells have been shown to produce more insulin in 3D collagen scaffolds. Unfortunately, 3D culture systems can prevent some cells from receiving sufficient oxygenation; this problem coupled with the beta cells high demand for oxygen can result in cell death due to hypoxia. The addition of an oxygen-producing biomaterial to a 3D culture has been found to be an effective method of providing additional oxygen to the cells. It was hypothesized that beta cell survival, growth, and function in vitro could be improved by culturing them in a 3D collagen scaffold with the addition of an oxygenating biomaterial (a PDMS-H$_2$O$_2$ disk). This hypothesis was tested through the use of COMSOL Multiphysics software to produce simulations of the culture environment. The simulations were performed using information gathered through experimentation, mathematical modeling, and calculation. Three different experiments were conducted using COMSOL: constant cell density, enhanced disk, and cell growth simulations. Through these three experiments it was found that PDMS-H$_2$O$_2$ disks could improve the oxygenation of a cell culture. The ideal culture conditions were found to be a cell culture with two PDMS-H$_2$O$_2$ disks, and an initial cell density of less than 500,000 cells.
**Introduction**

Millions of people around the world are currently suffering from diabetes, and the prevalence of the disease is increasing by approximately 2-5% each year (2). Studies on the disease have predicted that over 300 million people worldwide will be diagnosed with diabetes by the year 2025. (9) The group of metabolic diseases known as diabetes all relate to the inability of the body to regulate blood glucose levels because of abnormalities with the production or effectiveness of the hormone insulin. (4) Insulin is secreted by pancreatic beta cells in response to high blood glucose levels. The body’s failure to regulate blood-glucose level can result in severe complications such as kidney failure, cardiovascular disease, blindness, amputation, coma and death. (2). The two most common types of diabetes are referred to as type I and type II diabetes. Both, type I and type II diabetes inhibit a patient’s ability to regulate blood glucose, but they also differ in several ways. The onset of type I diabetes typically occurs in childhood or adolescence. Type I diabetes is caused by the autoimmune destruction of pancreatic beta cells, which results in the inability to produce a sufficient amount of insulin to meet the body’s needs. Type II diabetes usually occurs later in life, and is the result of the patient developing a physiological resistance to insulin. (2) Traditionally, diabetes is treated by daily injections of insulin, and by closely monitoring blood glucose levels (3). This method of treatment can be difficult for patients to maintain because it requires a dramatic lifestyle change, frequent doctor visits, and intensive monitoring of blood-glucose levels. The transplant of insulin-producing pancreatic beta cells into diabetic patients is a promising alternative to the traditional insulin therapy for the treatment of diabetes. Pancreatic tissue transplants are used to increase a patient’s beta cell mass and improve the patient’s ability to produce
insulin and regulate their own blood-glucose levels. A study by Shapiro et al demonstrated several successful pancreatic islet transplants in type 1 diabetic patients. The tissue used in pancreatic islet transplants most often comes from brain dead or cadaveric donors with the families consent. (8) However, there is a shortage of the donor tissue needed for the transplant of beta cells. This shortage has lead researchers to search for a new source of pancreatic tissue. Currently, researchers are experimenting with the possibility of mass-producing biologically functional beta cells in vitro. (11) To this point, the mass-proliferation of beta cells in vitro has proved to be challenging for several reasons. Beta cells proliferate extremely slowly in vitro, and artificially elevating the proliferation rate can cause the beta cells to lose their original biological functions including the ability to produce insulin. (9). Another issue with culturing beta cells in vitro is that during insulin secretion, beta cells consume large amounts of oxygen. The beta cells will suffer from hypoxia and die if these oxygen demands are not met. (7) It has been found that the addition of oxygen-producing biomaterials to cells cultured in a 3D agarose scaffold can improve MIN6 beta cell viability. (6) Also, research has shown that beta cells that are cultured in a three-dimensional collagen scaffold tend to secrete more insulin than beta cells cultured in other conditions. (12) We hypothesized that beta cells may be more effectively proliferated in vitro by culturing them in a three-dimensional collagen-based culture environment, and by adding an oxygenating biomaterial to the environment. To test this hypothesis we used COMSOL Multiphysics software to generate mathematical models for simulations, which were designed to test the effectiveness of an oxygenating biomaterial (PDMS-H$_2$O$_2$) in a 3D collagen scaffold seeded with mouse beta cells. The Multiphysics simulations provided a means to optimize the culture conditions, and find the
culture conditions that are most likely to encourage cell survival. With the growing prevalence of debilitating disease that is diabetes, research in this area is crucial. Pancreatic islet transplantation could greatly improve a diabetic patients quality of life, but it is currently not a viable treatment of diabetes because of the shortage of donor tissue. If an effective method of generating insulin-producing pancreatic tissue in vitro were found, there would be no shortage of the tissue needed for transplantation. This research is an important step in the direction of improving the lives of patients suffering from diabetes everywhere.

**Materials and Methods**

**PDMS-H$_2$O$_2$ Disks**

It was hypothesized that an oxygen-producing biomaterial could be used to provide the additional oxygen needed by the beta cells in a 3D collagen scaffold. Dr. Sha Jin’s lab has been collaborating with a research group at The Ohio State University to fabricate an oxygen-releasing disk that is suitable for beta cell culture. The disk consisted of hydrogen peroxide (H$_2$O$_2$) encased within polydimethylsiloxane (PDMS). The PDMS-H$_2$O$_2$ disks used in experimentation had a 15% H$_2$O$_2$ concentration. Each of the disks used were 7mm in diameter with a thickness of 1.5mm. When hydrogen peroxide reacts with the water (H$_2$O) in the culture media, oxygen (O$_2$) is produced. When hydrogen peroxide is encased within PDMS, PDMS (a hydrophobic polymer yet still partially permeable to water) regulates the reaction between hydrogen peroxide and water, and allows for sustained oxygen production. (6) In order to accurately model the production of oxygen using
COMSOL, the release kinetics of the oxygenating disks had to be measured through experimentation.

**Oxygen Release Kinetics Measurement**

A simple experiment was devised to measure the oxygen release kinetics of the oxygenating disks. First, a test tube was filled with 30ml of PBS buffer solution, and then five oxygenating disks were added to the solution. Before the five disks were added to the PBS buffer, all of the oxygen from the solution and the test tube that contained the solution was completely removed by using a gas manifold system and an anaerobic glove chamber. Using the NeoFox Phase Measurement System, the oxygen concentration within the PBS buffer solution with the five oxygenating disks added was recorded once per hour over a period of 18 days. Figure 1A shows the increase in oxygen concentration (μ mol/L) in the PBS buffer over time (seconds). The oxygen release rate of the disks was calculated at each time point using the data shown in Figure 1A, and then plotted against the change in oxygen concentration as shown in Figure 1B.
Figure 1: Figure 1A shows the increase of the oxygen concentration in the PBS buffer solution over time. A logarithmic trend line was added to the graph of the data. The resulting logarithmic equation was used to find the rate of oxygen production at each concentration data point by finding the derivative of the logarithmic equation and plugging in the concentration values that were gathered once per hour. Using the oxygen release rates, \( V_{max} \) was found to be 2.57e-6 mol/ (m^3*s). Figure 1B shows the relationship between oxygen production rate and oxygen concentration. A logarithmic trend line was added to the data. This data was used to find that the reactions \( K_m \) was equal to 5.34e-5 mol/m^3.
The results in Figure 1 provided important data that was needed to continue the experiment. Figure 1A shows that the five oxygenating disks caused an increase in oxygen concentration in a closed system with no oxygen initially. The increase in oxygen demonstrates that these oxygenating disks could be used to increase the oxygen concentration of a 3D cell culture scaffold. The data also provided information about the reaction kinetics of the oxygen release from the oxygen-producing disks. This information allowed for the calculation of the maximum production rate ($V_{\text{max}} = 2.572 \times 10^{-6} \text{ mol/ (m}^3\text{*s)}$) and the reaction constant ($K_m = 5.34 \times 10^{-5} \text{ mol/m}^3$) of the oxygen release reaction that occurs within one of the oxygenating disk. These values were input into the COMSOL simulations in order to model oxygen production and release from the disks.

**Formation of Oxygen Release Equation**

The oxygen release reaction was assumed to behave similarly to Michaelis-Menten reaction kinetics after analyzing the data gathered during the experiment. Equation 1 (see below) was used to model the release of oxygen from the PDMS-$\text{H}_2\text{O}_2$ disks. In Equation 1, $V_{O_2}$ represents the release rate of oxygen from the disk (mol/ (m$^3$*s)), $V_{\text{max}O_2}$ represents the maximum rate of the oxygen production reaction (mol/ (m$^3$*s)), $C_{\text{H}_2\text{O}_2}$ is the concentration of $\text{H}_2\text{O}_2$ within the oxygenating disk, and $K_{mO_2}$ represents the reaction constant in the reaction. As mentioned above, the $K_{mO_2}$ and $V_{\text{max}O_2}$ were measured during experimentation. The value $K_{mO_2}$ for the disks was found to be $5.34 \times 10^{-5} \text{ mol/m}^3$, and the value of $V_{\text{max}O_2}$ was found to be $2.572 \times 10^{-6} \text{ mol/ (m}^3\text{*s)}$. Equation 1 with these values was considered the standard for oxygen production kinetics for all simulations containing oxygenating disks. In an attempt to enhance the oxygen production capabilities
of the oxygenating disk, the $V_{max_{O2}}$ was suggested to increase in some of the simulations performed by optimizing the fabrication of oxygen-release disk.

$$V_{O2} = \frac{V_{max_{O2}} C_{H2O2}}{C_{H2O2} + Km_{O2}} \quad (1)$$

**Formation of Oxygen Consumption Equation**

The oxygen consumption equation (Equation 2) used was selected based on research performed in similar studies. Buchwald et al and Pedraza et al both modeled oxygen consumption of beta cells using Michaelis-Menten reaction kinetics. (1)(6) In Equation 2 below, $OCR$ represents the oxygen consumption rate of the beta cells; $OCR_{max}$ represents the maximum rate at which the beta cells will consume oxygen; $C_{O2}$ is the concentration of oxygen in the culture; and $Cmm_{O2}$ is the Michaelis-Menten reaction constant for the consumption of oxygen by beta cells.

$$OCR = \frac{OCR_{max} C_{O2}}{C_{O2} + Cmm_{O2}} \quad (2)$$

The $OCR_{max}$ was approximated as $6.367e-17 \text{ mol/(s*cell)}$ using data from a study by Papas et al. (5) However, COMSOL required the reaction units to be in $\text{mol/ (m}^3\text{*s)}$ so the $OCR_{max}$ was converted by multiplying the $OCR_{max}$ by the number of cells in culture over the volume of the container. Using this method the $OCR_{max}$ values for cultures placed within a 48-well plate and containing 500,000 cells, 1 million cells, and 2 million cells were found to be $7.8e-5 \text{ mol/(m}^3\text{*s)}$, $1.56e-4\text{mol/(m}^3\text{*s)}$, and $3.12e-4\text{mol/(m}^3\text{*s)}$, respectively. For MIN6 beta cells $Cmm_{O2}$ was determined to be $.005 \text{ mol/m}^3$ in a study by Pedraza et al. (6)
Construction of the Simulations

To accurately represent the 3D collagen culture system with COMSOL, several assumptions and parameters were used in the construction of the simulations. To model the production, convection, diffusion and consumption of oxygen in the culture system, COMSOL’s Transport of Diluted Species module was used for each simulation generated. Each culture simulation was assumed to take place in a single well of a 48-well plate (diameter=11.05mm, height=4.25 mm). In Figure 2 below, the 48-well plate is represented by the larger rectangle.

![Figure 2: The basic geometry of the culture system, where a 3D scaffold (d=11.05mm, h=4.25 mm) is placed in one well of a 48-well cell culture plate with a PDMS-H2O2 disk (d=7mm, h=1.5mm).](image)

The 48-well plate was assumed to contain a collagen scaffold with a uniform distribution of beta cells (regardless of the cell count). The collagen scaffold contained a collagen concentration of 1.5 mg/ml, and a water concentration of approximately 40 mol/m³. The collagen scaffold was assumed to have an oxygen diffusion coefficient of 2.99e-9 m²/s (10). The bottom, left, and right boundaries of the culture medium were set as closed boundaries (no flux of any species in or out), however the top boundary was modeled as having an oxygen concentration of 0.2 mol/m³ to represent atmospheric oxygen. The oxygenating disk is represented by the smaller rectangle within the 48-well plate. All of the simulations of the oxygenating disks were assumed to have an oxygen
diffusion coefficient of $6 \times 10^{-9}$ m$^2$/s and a water diffusion coefficient of $2.49 \times 10^{-9}$ m$^2$/s. (6) In the model system, a thin diffusion barrier (with a thickness of $5 \times 10^{-6}$m) was placed around all the boundaries of each simulated oxygenating disk to prevent the flow of H$_2$O$_2$ into the culture (oxygen and water were allowed to flow freely). The entire scaffold, including the oxygenating disk, was assumed to have an initial oxygen concentration of 0.2 mol/m$^3$.

Two reactions modeled in the simulations: production of oxygen and consumption of oxygen. The larger rectangle (the culture medium with cells seeded) was assumed to uniformly consume oxygen using Equation 2, and the smaller rectangle (the oxygen-producing disk) was assumed to uniformly produce oxygen using Equation 1. The mesh selected for every simulation was set to a normal (referring to the fineness or coarseness of the mesh) physics-controlled mesh.

**Interpretation and Analysis of Simulations**

After the completion of each COMSOL simulation, the data gathered was analyzed to determine the effectiveness of the PDMS-H$_2$O$_2$ as an oxygenator. COMSOL displays the oxygen distribution within in the culture using a rainbow color pattern in which, dark red represents high oxygen concentration (the maximum was set to 0.2 mol/m$^3$) and dark blue represents an oxygen concentration of 0 mol/m$^3$ or less. COMSOL also provides the user with the values of the highest and lowest oxygen concentration in the culture.

Buchwald et al determined that the beta cells would die from hypoxia if the oxygen concentration in the culture falls below $1 \times 10^{-4}$ mol/m$^3$ (this number is referred to as the critical oxygen concentration). (1) A designed culture system was considered successful if the results of the simulation showed that the area of the culture with lowest oxygen concentration was oxygenated significantly above the critical oxygen concentration.
Culture conditions in which certain portions of the culture media fell below or near the critical oxygen concentration were not recommended.

**Results and Discussion**

**Constant Cell Density Simulations**

In the first series of simulations, which were generated using COMSOL Transport of Diluted Species module, it was assumed that the beta cell density in the 3D collagen culture would remain constant during a short period of culture time. These simulations were performed to determine the culture sizes that would benefit the most from additional oxygenation. Three different beta cell densities were tested, including 500,000 cells per culture, 1 million cells per culture and 2 million cells per culture. For each different beta cell density, there were three simulations performed: a control beta cell culture with no oxygenating disk; a beta cell culture containing one oxygenating disk; and a beta cell culture containing two oxygenating disks. It was assumed that the beta cells would be constantly consuming oxygen at a rate based on the consumption kinetics defined in the materials and methods section. The oxygenating disks were modeled to produce oxygen using Equation 1. The initial conditions of all the simulations performed are the same as discussed in the materials and methods section. The simulations were allowed to run for a simulated time period of 10,000 seconds, at which point the system typically reached equilibrium (when oxygen distribution stopped noticeably changing).

Figure 3A demonstrates that a cell culture of 500,00 beta cells can receive enough oxygen from the environment alone for the entire culture to stay slightly above the critical oxygen concentration (1e-4 mol/m³). However, without an oxygenating disk in the
culture the oxygen concentration in the bottom of the 48-well plate is still very low, and the culture could potentially benefit from the addition of an oxygenating disk. Figure 3B (one oxygenating disk) and Figure 3C (two oxygenating disks) both show that the addition of one or more oxygenating disks to a cell culture containing 500,000 cells can oxygenate the cell culture to a point well above the critical oxygen concentration, and therefore increase the chances of cell survival.
Figure 3: This figure shows cell cultures seeded with 500,000 MIN6 beta cells (OCR\text{max}=7.8e-5 mol/(m^3*s)). Figure 3A is a cell culture without additional oxygenation. Although the minimum oxygen concentration (8.008e-3 mol/m^3) is above the critical oxygen concentration of 1e-4 mol/m^3 (the concentration of oxygen at which cells begin to die from hypoxia), the oxygen concentration in the bottom of the 48-well plate is very low. Figure 3B is a cell culture with one oxygenating PDMS-H\textsubscript{2}O\textsubscript{2} disk. The disk improved the overall oxygenation of the cell culture. The minimum oxygen concentration for this culture is .0398 mol/m^3, which is well above the critical oxygen concentration. Figure 3C is a cell culture containing two PDMS-H\textsubscript{2}O\textsubscript{2} oxygenating disks. The two disks provide excellent oxygenation of the entire cell culture with a minimum oxygen concentration of .079 mol/m^3.

As shown in Figure 4A, beta cell cultures with cell counts of 1 million cells or more were found to require supplemental oxygenation, in order to prevent hypoxia. The portion of the cell culture exposed to the open air will receive sufficient oxygen, but the cells located towards the bottom of the 48-well plate will be starved of oxygen. One oxygenating disk does significantly improve the oxygenation of the scaffold, but the oxygen demand of 1 million beta cells is more than one standard oxygenating disk can fully compensate for (Figure 4B). When the 1-million cell culture was modeled with two oxygenating disks, the oxygen concentration was high enough to overcome the critical oxygen concentration, and minimize the risk of hypoxia. However, the oxygen concentration is still very low in some regions of the scaffold (Figure 4C).
Figure 4: This figure shows cell cultures seeded with 1 million MIN6 beta cells (OCR$_{\text{max}}$=1.56e-4mol/(m$^3$\cdot s)). Figure 4A is a cell culture without additional oxygenation. A large portion of this cell culture is below the critical oxygen concentration; therefore many of the cells will die due to hypoxia. Figure 4B is a cell culture with one oxygenating PDMS-H$_2$O$_2$ disk. The disk improved the overall oxygenation of the cell culture. However, the minimum oxygen concentration (6.e-5 mol/m$^3$) for this culture is still well below the critical oxygen concentration. Figure 4C is a cell culture containing two PDMS-H$_2$O$_2$ oxygenating disks. The minimum oxygen concentration of this culture is 1.7e-3 mol/m$^3$, which shows the two disks provide sufficient oxygenation to the cell culture to prevent cell death due to hypoxia.
In the third group of simulations performed it was assumed that the 48-well plate was seeded with 2 million cells. Atmospheric oxygen on its own was not enough to meet the oxygen demands of the 2 million beta cells (Figure 5A). Also, in spite of increasing the oxygen concentration slightly, neither the one disk nor two disk simulations showed that the additional oxygenation would be able to meet the high oxygen demand of the 2 million cells (Figure 5B and Figure 5C).
Figure 5: This figure shows cell cultures seeded with 2 million MIN6 beta cells (OCR\textsubscript{max}=3.12e-4mol/(m\textsuperscript{3}*s)). Figure 5A is a cell culture without additional oxygenation. Almost all of this cell culture is below the critical oxygen concentration; therefore most of the cells will die due to hypoxia. Figure 5B is a cell culture with one oxygenating PDMS-H\textsubscript{2}O\textsubscript{2} disk. The disk very slightly improved the oxygenation of the cell culture. However, most of the cell culture is still below the critical oxygen concentration. Figure 5C is a cell culture containing two PDMS-H\textsubscript{2}O\textsubscript{2} oxygenating disks. The two disks only improve oxygenation of the cell culture by a small margin; therefore many cells will be lost due to hypoxic conditions.

Based on the constant cell density simulations, it was determined that the ideal culture size would be 500,000 beta cells per 48-well plate. With a culture size of 500,000 cells, most of the culture can survive using only atmospheric oxygen, and the addition of oxygenating disks can provide additional oxygen that will further minimize the risk of hypoxia. These results showed that it would be difficult or impossible to prevent hypoxia in a cell culture larger than that of 500,000 beta cells, even with the addition of multiple oxygenating disks.

Enhanced Disk Simulations

The results of the constant cell density simulations showed that a single oxygenating disk, that releases oxygen based on the reaction kinetics measured during experimentation, cannot provide enough oxygen to keep a culture of 1 million beta cells from suffering from hypoxia. Because of this result the next series of simulations were
constructed to determine if a single disk with enhanced oxygen release kinetics could meet the oxygen demand of the 1 million beta cells. The enhanced disk simulations were assumed to have a constant cell count of 1 million cells, and that the cells are constantly consuming oxygen. For each simulation the Vmax of the disk was increased to enhance the release of oxygen. A control simulation of a culture with 1 million cells seeded and a standard oxygenating disk was performed first (Figure 6A). The subsequent simulations (see Figures 6B, 6C, and 6D) contained oxygenating disks that were modeled to produce oxygen with Vmax values 10, 20 and 30 times higher than normal, respectively.

**Figure 6:** This figure shows cell cultures seeded with 1 million MIN6 beta cells (OCRmax=1.56e-4 mol/(m^3*s)). Figure 6A contains a standard oxygen producing PDMS-H2O2 disk that produces oxygen at the rate that was measured during experimentation (Vmax=2.572e-6 mol / (m^3*s)). This disk on its own is not capable of fully oxygenating a culture of this size. Figure 6B contains an enhanced PDMS-H2O2 that has a Vmax=2.572e-5 mol/(m^3*s). Although this disk releases oxygen at a faster rate, the disk itself does not provide sufficient oxygenation to the culture. Figure 6C this culture contains a further-enhanced disk that has a Vmax=5.144e-5 mol/(m^3*s). This disk was also unable to achieve a minimum oxygen concentration greater than the critical oxygen concentration. Figure 6D with a Vmax of 7.7116e-5 a single enhanced oxygen-producing disk was able to reach a minimum oxygen concentration that was greater than the critical oxygen concentration and therefore is capable of preventing hypoxic cell death.

Despite the increase in Vmax, the oxygenation did not improve in the cultures containing the oxygenating disk with a 10 or 20 times normal Vmax. The culture aided by
the disk with a Vmax 30 times greater than normal was able to reach a minimum oxygen concentration above the critical oxygen level. Unfortunately, the oxygen-producing capabilities of the oxygenating disk must be greatly altered to achieve a Vmax that is 30 times faster than the current oxygenating disk. Overall, enhancing the oxygen-release kinetics of a single oxygenating disk seemed to have little effect on the oxygenation of the cell culture. Also, it would be very difficult or impossible to produce an oxygenating disk, outside of a simulation, that had a sufficiently high Vmax that would significantly improve the oxygenation of the culture.

**Cell Growth Simulations**

The final series of simulations performed were intended to model an oxygenation-aided culture system, and to account for the effect of cell growth (but not cell death) on the oxygen demand of the culture over time. In the two simulations that were constructed, it was assumed that the beta cell count was initially 500,000. The constant cell density simulations demonstrated that cultures with 500,000 beta cells seeded are ideal because the oxygen needs of the cells can easily be met with the combination of atmospheric oxygen and one or two oxygenating disks. In order to account for cell growth in the culture, an exponential growth equation was used in conjunction with the oxygen consumption equation used previously. Based on our experimental results in the lab, the doubling time of MIN6 cells is every 64 hours. As seen in Figures 7 and 8, the oxygen distributions of the two cell growth simulations performed were recorded at four time points: 7000 seconds (apx. 2 hours), 129,000 seconds (apx. 36 hours), 259,000 seconds (apx. 72 hours), and 518,000 seconds (apx. 144 hours). The first simulation contained only one standard oxygenating disk. Although, the disk was able to sufficiently oxygenate
the culture initially, the growing oxygen demand quickly overwhelmed the oxygen produced. This fact would result in many cells in the culture being starved of oxygen as early as 36 hours into the culture.

**Figure 7:** This figure shows a cell culture initially seeded with 500,000 MIN6 cells. Exponential cell growth, with the MIN6 cells doubling every 64 hours, was assumed to occur. To aid the oxygenation of the culture one PDMS-H$_2$O$_2$ disk was added. Figure 7A shows the oxygen concentration distribution after approximately 2 hrs of culture. The oxygen-producing disk is able to fully oxygenate the culture at this time. Figure 7B shows the same culture after approximately 36hrs have passed. The minimum oxygen concentration at this time falls below the critical oxygen concentration, therefore hypoxic cell death will occur. Figure 7C The MIN6 culture after 72 hours. Most of the cells in the bottom of the 48-well plate will experience hypoxia leading to cell death. Figure 7D the same cell culture after 144 hrs has passed. Almost all of the MIN6 cells are now subject to hypoxic conditions.

The second simulation was constructed as a culture system aided by two standard oxygenating disks. At two hours and 36 hours into the culture the two oxygenating disks are able to provide enough oxygen to the cells to minimize the risk of hypoxia. However, after 72 hours of cell growth the two disks will no longer be able to support the beta cells oxygen demand.
Figure 8: This figure shows a cell culture initially seeded with 500,000 MIN6 cells. It was assumed that exponential cell growth with the MIN6 cells doubling every 64 hours. To aid the oxygenation of the culture two PDMS-H2O2 disks were place in the culture. Figure 8A the culture after approximately 2 hrs has passed. At this time the disks are able to fully oxygenate the cell culture. Figure 8B after approximately 36 hrs have passed; the disks are still able to meet the O2 demands of the MIN6 cells. Figure 8C shows the MIN6 culture after 72 hrs. At this time point the cell culture has grown too large for the disks to be able to fully support the culture. Figure 8D at 144 hrs after the initial culture, most of the cells 48-well will suffer from hypoxia.

Conclusion

The three different types of simulations (constant cell density, enhanced disk and cell growth simulations) helped determine the effectiveness of PDMS-H2O2 disks in providing additional oxygenation to a beta cell culture. The constant cell density simulations demonstrated that cultures with beta cell densities of 1 million cells or greater will suffer from severe hypoxic conditions even if supported by an additional oxygenator. Cultures with 500,000 beta cells were shown to benefit significantly from the addition of oxygen-producing disks. In these cultures, oxygenating disks raised the overall oxygen concentration, and minimized the chance of cell death due to hypoxia. The results of the enhanced disk simulations showed that enhancing the maximum oxygen release rate of the oxygenating disks could only improve the overall oxygenation of the culture if the
maximum release rate was over 30 times larger than the standard maximum release rate. It is unlikely that the production of such a disk in reality would be possible. Based on the results from the cell growth simulations, it has been determined that 500,000 beta cells is too large of an initial cell count. The culture will quickly grow to a size that cannot be helped by the addition of one or even two oxygenating disks. However, if the initial conditions of the cell culture were adjusted it could lead to a culture that would last much longer, but contains a smaller and more manageable number of cells. Overall, it was found under the right culture conditions, the addition of a PDMS-H$_2$O$_2$ would be beneficial to a beta cell culture within a 3D collagen scaffold. To confirm the simulation results obtained by the mathematical modelling, other members in our lab have examined the effectiveness of PDMS-H$_2$O$_2$ disks as an oxygenator for mouse beta cell cultures. When seeding 253,000 cells in 3D collagen culture system in a well of 48-well plate, we found that beta cells are able to secrete much more insulin compared to the cells cultured in the absence of oxygenator (data not shown), suggesting the improvement of functional beta cell culture. In the future, research could focus on the use of human beta cells as opposed to merely using mouse beta cells. The mathematical models of the 3D beta cell culture system could also be improved upon by adjusting equations, initial conditions, boundary conditions, and values to be more “life like” through the collection of more data on the release of oxygen from PDMS-H$_2$O$_2$ disks, the consumption of oxygen by beta cells, and the properties of a 3D collagen scaffold. Although the prospects of developing a source of insulin-producing beta cell tissue is promising, much more research is needed to find the optimal culture conditions to grow the human beta cells that are needed for transplant into diabetes patients.
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