Secretion of Organic Compounds from Tissue Engineered Adipose on Silk Scaffolds of Differing Sizes Compared to Differing Culture Periods

Brooklyn Grossbard

Nyack High School

Introduction

The medical field currently relies on many implantable devices that require electric energy to run. For example, each year 600,000 pacemakers are implanted worldwide (Wood, 2002). Currently, pacemakers and other implantable medical devices are powered by lithium-iodide or lithium anode cells (Olshansky, 2011). Unfortunately, the batteries upon which these devices rely have to be changed every so often, which requires surgery. Biofuel cells would eliminate the need for battery replacement by allowing the implanted device to work independently of an outside fuel source by using the patient's own body as a source of energy.

Unlike batteries, fuel cells can produce energy for as long as there is a fuel supply. Fuel cells use a fuel to convert chemical energy into electricity through oxidation. The three main parts of fuel cells are the anode, the cathode, and the catalyst. At the anode, a catalyst oxidizes the fuel, and the lost electron is sent to the cathode; the site of reduction, where the electron is gained. Biofuel cells work similarly to other fuel cells, with the exception of their catalyst. Biofuel cells use enzymes as catalysts as opposed to metals because they are organic and easier to produce in large quantities (Rasmussen, 2012). Additionally, the ease at which these enzymes can be produced allows the potential cost of the biofuel cell to be less than a fuel cell that uses nickel or platinum as a catalyst.

Research has shown that biofuel cells using glucose have been successful in producing electricity when implanted in insects, clams, and lobsters (Zebda, 2013). A study by MacVittie found that multiple lobsters implanted with biofuel cells could power one watch (2012). The largest success for biofuel cells so far came in 2013 when glucose biofuel cells directly implanted in rats were able to use the rat's bodily fluids as the only source of power for a light-emitting diode (LED) without rejection or infection. The cells used the glucose that is secreted from adipocytes as fuel. Glucose oxidase, an enzyme, converted glucose into gluconolatone by removing an H^+ ion (Zebda, 2013).

Fat is the largest energy reserve in the human body. Adipose tissue, or body fat, is a loose connective tissue. The majority of cells that make up adipose are adipocytes. These fat cells are characterized by their large lipid droplet size (Horowitz, 2003). The majority of the fat we consume is in the form of triglycerides. The triglycerides are broken down into smaller droplets of triglyceride, and then in the pancreas lipases break down the triglyceride into glycerol and fatty acids. These smaller parts are able to enter the cells that line the intestine where they are reassembled into packs of triglycerides called chylomicrons. The chylomicrons enter the blood stream, but do not last long, and are finally stored as triglyceride, glucose, and fatty acids in

the adipose cell. When the adipocytes break down, these organic compounds are released and used as energy (Freudenrich, 2012).

Implantable medical devices could be powered by a biofuel cell that uses the organic compounds that are secreted when adipocytes break down as the catalyst ~~fuel for the cell~~. If successful, the biofuel cell would first use fuel from organic compounds that are secreted from tissue-engineered adipocytes, but over time the patient's own adipocytes would take over. A structure, or scaffold, could be made out of a biomaterial and then seeded with adipocytes, which in turn would proliferate and cover the scaffold. This scaffold would be implanted into the patient along with the biofuel cells.

Silk Fibroin is a commonly used biomaterial due to its high biocompatibility and mechanical properties (Kaplan, 2011). This means when it is implanted there is very little risk of rejection, and the scaffold can dissolve without an issue. Silk fibroin sponges are most commonly used for scaffolds. These silk fibroin sponges have been used in the creation of bone, cartilage, soft tissue, and vascular tissue, to name a few (Wray, 2012).

The first step to creating a biofuel cell for use in implantable medical devices is to develop a process to engineer human adipocyte cells. The purpose of this project was to determine the feasibility of engineering adipocyte cells that secrete concentrations of organic compounds that will be beneficial to the biofuel cell. Adipocytes were grown on silk fibroin scaffolds of different sizes and cell samples were collected at a variety of stages~~days~~. The samples were analyzed for the concentration of triglyceride, free fatty acid, and glucose. These results will be used to optimize the scaffold size and cultivation time to achieve maximum fuel potential.

Methodology

Silk Scaffolds

Silk Fibroin Preparation

Silk fibroin scaffolds were prepared using a similar protocol to that conducted by Meinel, et al. (2009). First, steamed silk cocoons were cut into dime-size pieces. Two liters of distilled water were brought to a boil and enough sodium carbonate to prepare a 0.02 M solution of Na_2CO_3 was added and allowed to dissolve. Next, the cocoon pieces were added to the solution and kept in the boiling water, with consistent stirring, for 30 minutes. This caused the sericin to dissolve, leaving only silk fibroin. The silk fibroin was then rinsed in cool distilled water three times. Then, the fibroin was placed in a beaker filled with one liter of distilled water and a stir bar. The silk fibroin was stirred in the water for 20 minutes on a gentle stir setting. This process was carried

out a total of three times. The silk was then wrung out, spread out on aluminum foil, and allowed to dry in a fume hood overnight. Afterwards, the silk was dissolved in lithium bromide. A 9.3 molar LiBr solution was prepared. The silk fibroin was packed into a glass beaker and the LiBr solution was added on top. The fibroin with the LiBr was allowed to dissolve in an oven at 60˚C for four hours. Finally, the LiBr was removed from the fibroin through dialysis.

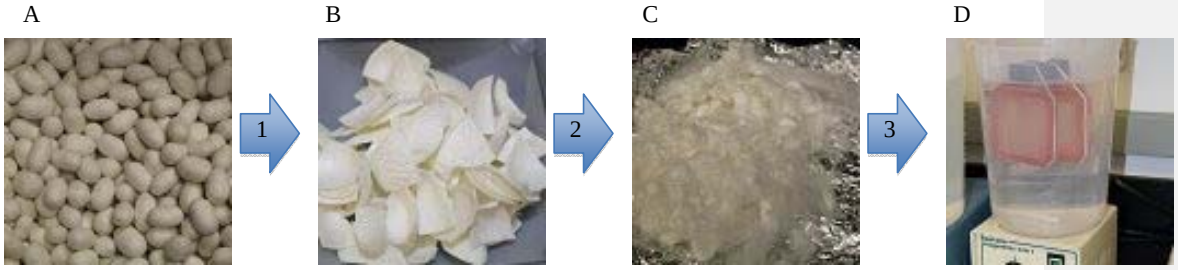

Figure 1: A: Silk cocoons from *bombyx mori* silk worms.
B: Silk cocoons cut into dime sized pieces
C: Silk fibroin after the sericin has been removed using Na_2CO_3
D: Silk fibroin during dialysis to remove the LiBr added to liquefy the silk
Photos taken by Brooklyn Grossbard

Scaffold Preparation

Four grams of salt were placed in a weigh boat; one for each scaffold. Next, two ml of silk fibroin solution (per scaffold) were syringed into plastic containers. The salt was then slowly poured on top of the silk fibroin solution while rotating the container so as to properly distribute the salt throughout the solution. The containers were closed and tapped to remove air bubbles and then allowed to settle overnight. Once the silk had gelled, the lids were removed and the containers were placed in two liters of deionized water. The beaker with the water and the scaffolds was transferred to a stir plate and stirred. The water was changed three times per day for two days. Scaffolds were stored in deionized water in tubes at 4°C. The silk scaffolds were then cut to 2x4mm, 2x6mm, 2x8mm, 2x10mm, and 12x12mm. Finally they were autoclaved to sterilize.

Figure 2: a finished silk scaffold

Photo taken by Brooklyn Grossbard

Obtaining Adipocytes

All adipocytes were donated from liposuctions done by hospitals in the area surrounding Tufts University. Donors received a form informing them about what the cells would be used for.

Figure 3: Material donated from an abdominoplasty.

Photo by Brooklyn Grossbard

Seeding Lipoaspirate Containing Cells and Extracellular Matrix

The fat was dissected from the fascia of scarpa and the skin, then placed into a blender and blended in bursts until the fat was broken up but not completely liquefied. This was to make sure that the adipocytes were still intact and surrounded by the matrix. Next, a two ml pipette with the filter broken off and attached to the aspirator tube was used to pick up the scaffold. The scaffolds were placed in centrifuge tubes filled with the blended adipocytes according to size. The tubes were then incubated for one hour. After incubating the fat in the scaffolds, the scaffolds that contained the fat were poured into a sterile petri dish. Next, each scaffold was transferred to a well in a 24-well plate. The scaffolds were incubated for two hours to allow the cells to attach to the scaffolds. Then 1 ml~~l~~ of fetal bovine serum was added to each well. The medium was removed at days 3,7,10,18,21, and 28 for organic compound testing.

Figure 4: Adipose tissue before it's blended.

Photo by Brooklyn Grossbard

Assays

Assays were run to find the concentration of triglyceride, free fatty acid, and glucose in the medium. Assays were run for each of the scaffold sizes; 2x4mm, 2x6mm, 2x8mm, 2x10mm, and

12x12mm-,and for each of the days the medium was removed at; 3,7,10,18,21, and 28. The assays were all from assay kits with their own independent procedures. The triglyceride assay required a 5x dilution, and the glucose assay required a 1000x dilution. Both dilutions were done using phosphate buffer saline (PBS). The concentration, of triglyceride, free fatty acid, and glucose were then calculated and graphed.

Statistics

A two-way randomized ANOVA was used to measure the contribution of two independent variables, scaffold size and culture period, to the variance between and within the concentrations secreted with the different organic compounds. Each organic compound was tested for three null hypotheses: the effect of the culture period, the effect of the scaffold size, and the interaction of both. A p-value of less than .001 was considered significant.

Comment [m1]: Was it 0.001 or 0.01? In the results, you say 0.01

Formatted: Highlight

Results

Cultured Adipocytes

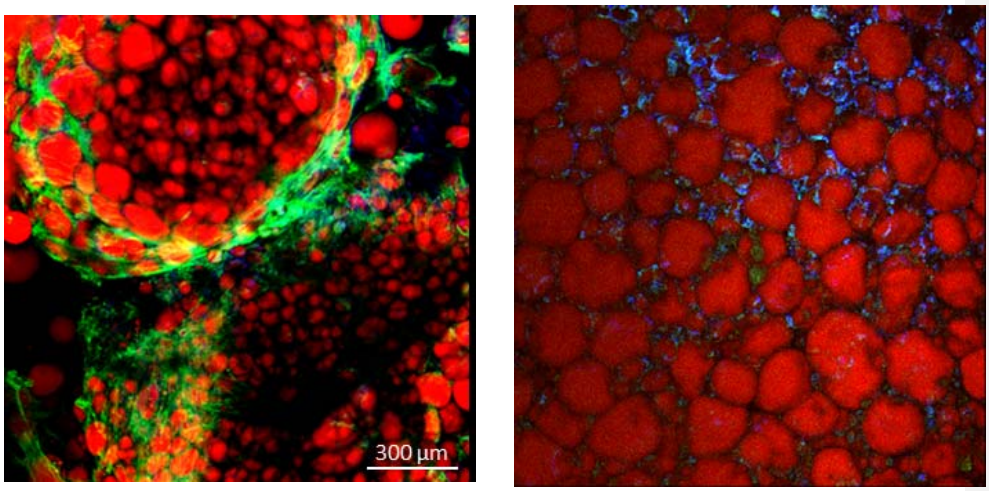

Figure 5: Shown above are images of seeded adipocytes on silk scaffolds obtained through confocal microscopy. These images were taken after the adipocytes had matured.

As can be seen in the images above, the scaffold and cells were stained in order to highlight different parts of the adipocytes. The blue staining is DAPI, which binds to regions

with high concentrations of A-T in DNA. The DAPI staining can also be seen as green in the images above. The red staining is AdipoRed, which is an Adipose Assay Reagent. Specifically speaking, it highlights the large lipid droplets, which characterize adipocytes. The purple color in the image is the silk. The combining of the dyes on the silk causes this purple fluorescence. The images above show that the adipocytes have fully infiltrated the scaffold and are proliferating and maturing comfortably. This is necessary for the results of the study because this proliferation is highly necessary for the secretion of the organic compounds, which were calculated and will eventually power the biofuel cell.

Comment [m2]: Is there another word to use here?

Formatted: Highlight

Glucose

The concentration of glucose in the fat medium was high, and so the medium had to be diluted 100x with PBS to ensure an accurate read.

Table 1: The table below shows the comparison of culture period to scaffold size for the concentration of glucose secreted. The number shown below each day is the concentration secreted in mmol/L. The average and standard deviation are shown for both the concentration for differing scaffold size when the culture period is constant, and the concentration for differing culture periods when scaffold size is isolated.

Size (mmxmm)	**Day 3**	**Day 7**	**Day 10**	**Day 18**	**Day 21**	**Day 28**	**Average**	**Standard Deviation**
2x4	0.165	0.184	0.213	0.095	0.222	0.113	0.165	0.468
2x6	0.119	0.078	0.103	0.102	0.057	0.105	0.094	0.022
2x8	0.121	0.202	0.089	0.113	0.094	0.169	0.131	0.045
2x10	0.208	0.090	0.228	0.114	0.114	0.175	0.115	0.057
2x12	0.134	0.116	0.156	0.117	0.293	0.117	0.164	0.066
Average	0.149	0.134	0.158	0.108	0.156	0.136	N/A	N/A
Standard Deviation	0.038	0.056	0.063	0.009	0.098	0.033	N/A	N/A

As shown in Ttable 1, the highest concentration secreted of glucose overall was 0.293 mmol/L. This amount was secreted on the 2x12mm scaffold after a culture period of 21 days. The lowest concentration secreted on the other hand was 0.057 mmol/L on the 2x6mm scaffold

after a culture period of 21 days. It is interesting to note that the lowest and highest secretion overall for glucose occurred with the same culture period because it points out the high effect the scaffold size has on concentration secreted. It can also be seen in ~~the~~ T~~t~~able 1 ~~above~~ that the culture period with the highest concentration secreted overall was 10 days. The average concentration secreted for a culture period of 10 days was 0.158 mmol/L. The standard deviation for the average concentrations secreted after a culture period of 10 days was 0.063. The most effective scaffold size was found to be the 2x4mm scaffold. The average overall concentration secreted for the 2x4mm scaffold was 0.165 mmol/L. It is important to note, however, that there was a large outlier for the 2x4mm scaffold concentration averages. After a culture period of 21 days the 2x4mm scaffold secreted 0.222 mmol/L of glucose, which was much higher than what the 2x4mm scaffolds were secreting with the other culture periods. The standard deviation for the average concentration secreted on the 2x4mm scaffolds was 0.468, which is much higher than the standard deviations for the other averages.

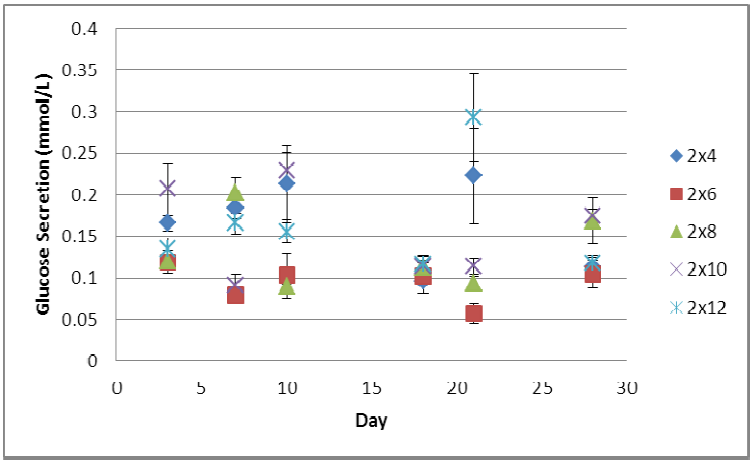

Figure 5: The graph above compares the concentration of glucose secreted in mmol/L to the cell culture period in days. Different shapes represent the different scaffold sizes, and error bars have been added.

Figure 5 compares the concentration of glucose secreted for the different scaffold sizes and culture periods. Because there was no apparent pattern, no line was added connecting the data points for scaffolds of the same size. Error bars were added in to show the actual possible

range given the error on each concentration found. It can be seen from ~~the graph~~ Figure 5 that often the concentration secreted for a particular scaffold size would increase as the length of culture period increased, until a certain culture period. At this point the concentration secreted would drop significantly, and then slowly begin the rise again. This may be caused by the cells proliferating to a point where there is no more room on the scaffolds, so many of them die off, which is when the process starts over again.

Table 2: A two way randomized ANOVA statistics test was run comparing the concentration secreted with the day of cell culture and the scaffold size.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Culture Period	0.064659933	4	0.016164983	33.0669932	1.45394E-14	2.525215102
Scaffold Size	0.024999656	5	0.004999931	10.22782917	4.03516E-07	2.368270235
Interaction	0.1668034	20	0.00834017	17.06060186	9.09746E-18	1.747984133
Within	0.029331333	60	0.000488856			
Total	0.285794322	89				

Comment [m3]: Can you get rid of the last column, since you don't discuss it?

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Table two shows the results from a two way randomized ANOVA test that was run on the found concentrations of glucose to compare the effects of culture period, scaffold size, and the interaction between the two on the overall concentration. A two-way randomized ANOVA analyzes the variance between groups and within groups. If either of the variables tested has an effect then the variance between the groups is greater than the variance within the groups. A two-way randomized ANOVA tests for three null hypotheses. ~~,~~ ~~t~~The null hypotheses in the ANOVA looked at the effect the two groups have on the concentration, and the effect on the concentration that they had together. A p-value of less than .01 was said to be significant. And any p-value less than .01 was said to reject the null hypothesis~~;~~ meaning that the group had a significant effect on the overall concentration.

As can be seen in ~~t~~Table 2, the p-values for the concentrations based on culture period, scaffold size, and the interaction between the two are significantly less than .01. To further analyze the significance of the results a Tukey's honestly signicant differece (HSD) was performed. Tukey's HSD test is used to determine exactly what groups are significantly different

from each other. The HSD found for the scaffold size differrence for a significance of <.01 was 0.017, meaning that concentrations that were greater than 0.017 mmol/L different were significant. Refering back to Ttable 1 the difference in average concentration between each scaffold size was greater than 0.017 as the scaffold sized increased. The HSD found for the difference in culture period with a significance of <.01 was 0.016 mmol/L. Again referring back to Ttable 1 the difference in average concentration between each culture period were significant between days 7 and 10, days 10 and 18, days 18 and 21, and finally days 21 and 28. This suggestsproves that changing the scaffold size and concentration has a real impact on the concentration of glucose secreted.

Free Fatty Acid

The free fatty acid assay required no dilution from the fat medium, so the working reagent was added to the medium with fat cells after different culture periods. Because no dilution was necessary, and the concentration was measure in µM as opposed to mmol/L, there tends to be a higher percent error for the concentration secreted

Table 3: The table below shows the comparison of culture period to scaffold size for the concentration of free fatty acid secreted. The number shown below each day is the concentration secreted in µM. The average and standard deviation are shown for both the concentration for differing scaffold size when the culture period is constant, and the concentration for differing culture periods when scaffold size is isolated.

Size (mmxmm)	**Day 3**	**Day 7**	**Day 10**	**Day 18**	**Day 21**	**Day 28**	**Average**	**Standard Deviation**
2x4	330.266	414.199	486.399	702.666	602.555	362.444	483.088	145.019
2x6	378.933	502.866	578.733	233.533	527.333	528.388	458.297	128.831
2x8	741.399	701.599	603.799	616.466	561.944	761.111	664.386	81.404
2x10	811.133	731.199	698.933	872.733	492.388	719.277	720.944	129.618
2x12	714.199	767.399	782.399	559.466	806.833	822.722	742.170	97.044
Average	595.186	623.452	630.053	596.973	598.211	638.788	N/A	N/A
Standard Deviation	223.166	155.535	113.922	235.101	123.571	189.623	N/A	N/A

As can be seen above in Ttable 3, the highest concentration secreted of free fatty acid overall was found to be 872.733 µM. This amount was secreted on the 2x10mm scaffold after a culture period of 18 days. The lowest concentration secreted on the other hand was 233.533 µM on the 2x6mm scaffold after a culture period of 18 days. It is interesting to note that the lowest and highest secretion overall for glucose occurred with the same culture period because it points out the high effect the scaffold size has on concentration secreted. It can also be seen in the table above that the culture period with the highest concentration secreted overall was 28 days. The average concentration secreted for a culture period of 28 days was 638.788 µM. The standard deviation for the average concentrations secreted after a culture period of 28 days was 189.623 µM. The most effective scaffold size was found to be the 2x12mm scaffold. The average overall concentration secreted for the 2x12mm scaffold was 742.170 µM with a standard deviation of 97.044 µM. This standard deviation is relatively low compared to the other standard deviations. It is also important to note that the culture period and scaffold size that were the most successful for the concentration of free fatty acid secreted were not the same as those that were the most successful for the concentration of glucose secreted.

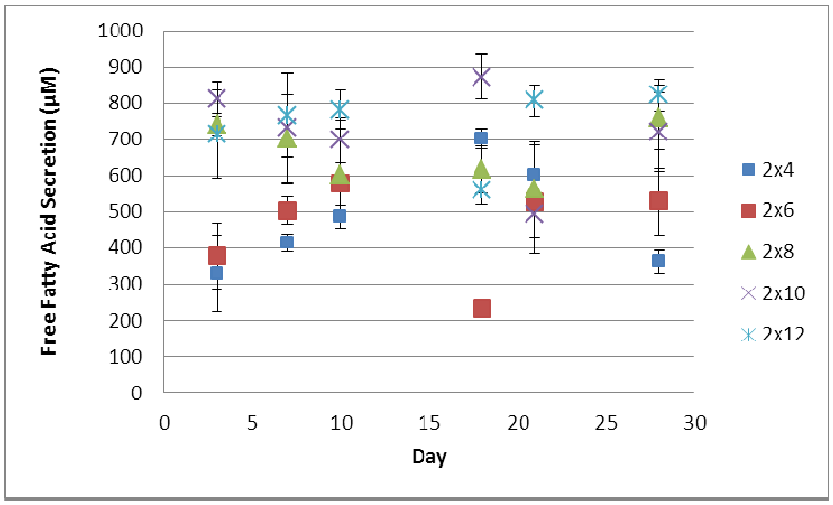

Figure 6: The graph above compares the concentration of free fatty acid secreted in µM to the cell culture period in days. Different shapes represent the different scaffold sizes, and error bars have been added.

Because there was no apparent pattern no line was added connecting the data points for scaffolds of the same size. Error bars were added in to show the actual possible range given the error on each concentration found. Similar~~ly~~ to the graph of glucose secreted, it can be seen from ~~the graph~~ Figure 6 that often the concentration secreted for a particular scaffold size would increase as the length of culture period increased, until a certain culture period. At this point the concentration secreted would drop significantly, and then slowly begin the rise again. This may be caused by the cells proliferating to a point where there is no more room on the scaffolds, so many of the die off, which is when the process starts over again.

Table 4: A two way randomized ANOVA statistics test was run comparing the concentration secreted with the day of cell culture and the scaffold size.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Culture Period	1462579.75	4	365644.936	17.8225157	1.08E-09
Scaffold Size	383986.120	5	76797.2399	3.74330363	0.005
Interaction	1189198.74	20	59459.9372	2.89823695	7.70E-04
Within	1230953.95	60	20515.8992		
Total	4266718.64	89			

Table four shows the results from a two way randomized ANOVA test that was run on the found concentrations of free fatty acid to compare the effects of culture period, scaffold size, and the interaction between the two on the overall concentration. As can be seen in T~~t~~able 4~~2~~ the p-values for the concentrations based on culture period, scaffold size, and the interaction between the two are significantly less than .01.

To further analyze the significance of the results a Tukey's honestly signicant differece (HSD) was performed. The HSD found for the scaffold size differ~~n~~ence for a significance of <.01 was 112.760 µM , meaning that concentrations that were greater than 112.760 µM different were significant. Referring back to T~~t~~able 3 the difference in average concentration between each scaffold size was greater than 112.760 between the larger and smaller sized scaffolds. The HSD found for the difference in culture period with a significance of <.01 was 105.333 µM. As culture period increased there was no significance found between concentrations secreted.

Triglyceride

The triglyceride was diluted 5x with phosphate buffer saline (PBS) in order to get a fluorescence, which was in an accurate range of the spectrophotometer.

Table 5: The table below shows the comparison of culture period to scaffold size for the concentration of triglyceride secreted. The number shown below each day is the concentration secreted in mmol/L. The average and standard deviation are shown for both the concentration for differing scaffold size when the culture period is constant, and the concentration for differing culture periods when scaffold size is isolated.

Size (mmxmm)	**Day 3**	**Day 7**	**Day 10**	**Day 18**	**Day 21**	**Day 28**	**Average**	**Standard Deviation**
2x4	0.302	0.446	0.572	1.213	0.854	0.401	0.631	0.343
2x6	0.711	1.322	0.745	1.100	1.809	0.542	1.038	0.473
2x8	0.961	1.531	1.215	1.542	1.633	1.025	1.318	0.289
2x10	1.628	1.878	1.393	0.400	1.322	0.647	1.211	0.573
2x12	1.334	1.518	1.029	0.706	1.403	0.701	1.115	0.358
Average	0.987	1.339	0.991	0.992	1.404	0.663	N/A	N/A
Standard Deviation	0.519	0.538	0.335	0.446	0.363	0.232	N/A	N/A

As shown in Ttable 5, the highest concentration secreted of triglyceride overall was 1.878 mmol/L. This amount was secreted on the 2x10mm scaffold after a culture period of 7 days. The lowest concentration secreted on the other hand was 0.302 mmol/L on the 2x4mm scaffold after a culture period of 3 days. It can also be seen in the table above that the culture period with the highest concentration secreted overall was 21 days. The average concentration secreted for a culture period of 21 days was 1.404 mmol/L. The standard deviation for the average concentrations secreted after a culture period of 21 days was 0.363. The most effective scaffold

size was found to be the 2x8mm scaffold. The average overall concentration secreted for the 2x8mm scaffold was 1.318 mmol/L with a standard deviation of 0.289.

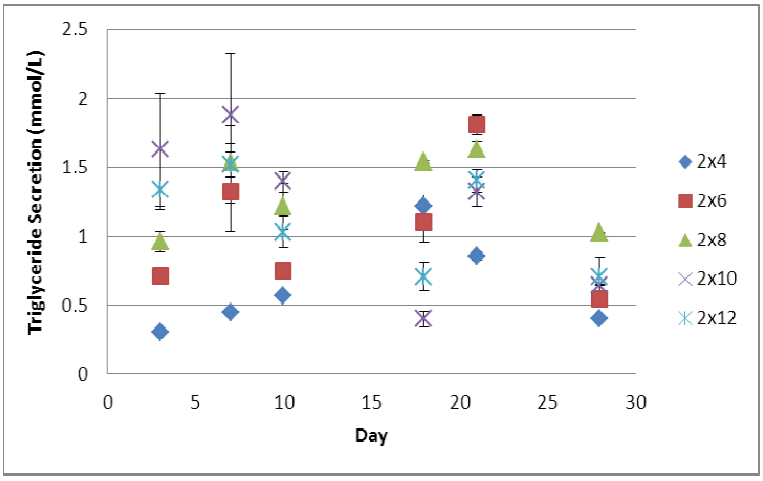

Figure 7: The graph above compares the concentration of triglyceride secreted in mmol/L to the cell culture period in days. Different shapes represent the different scaffold sizes, and error bars have been added.

Because there was no apparent pattern, no line was added connecting the data points for scaffolds of the same size. Error bars were added in to show the actual possible range given the error on each concentration found. It can be seen from Figure 7~~the graph~~ that often the concentration secreted for a particular scaffold size would increase as the length of culture period increased, until a certain culture period. At this point the concentration secreted would drop significantly, and then slowly begin the rise again. This may be caused by the cells proliferating to a point where there is no more room on the scaffolds, so many of the die off, which is when the process starts over again.

Table 6: A two way randomized ANOVA statistics test was run comparing the concentration secreted with the day of cell culture and the scaffold size.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Culture Period	5.26991703	4	1.3174793	26.701475	9.63E-13
Scaffold Size	5.41466351	5	1.0829327	21.947898	1.98E-12
Interaction	7.78625243	20	0.3893126	7.8902352	2.08E-10
Within	2.96046401	60	0.0493411		
Total	21.431297	89			

Table 6~~six~~ shows the results from a two way randomized ANOVA test that was run on the found concentrations of triglyceride to compare the effects of culture period, scaffold size, and the interaction between the two on the overall concentration. As can be seen in T~~t~~able 6~~six~~ the p-values for the concentrations based on culture period, scaffold size, and the interaction between the two are significantly less than .01.

To futher analyze the significance of the results a Tukey's honestly significant difference (HSD) was performed. The HSD found for the scaffold size differ~~n~~ence for a significance of <.01 was 0.175 mmol/L, meaning that concentrations that were greater than 0.175 mmol/L different were significant. Referring back to T~~t~~able 5 the difference in average concentration between each scaffold size was greater than 0.175 mmol/L between the 2x4mm, 2x6mm and 2x8mm scaffolds. The HSD found for the difference in culture period with a significance of <.01 was 0.163 mmol/L. As culture period increased there was significance between days 3 and 7, days 7 and 10, days 18 and 21, and days 21 and 28. This suggests~~proves~~ that changing the scaffold size and concentration has a real impact on the concentration of triglyceride secreted.

Discussion

The purpose of this project was to determine the feasibility of engineering adipocyte cells that secrete concentrations of organic compounds that ~~will be beneficia~~could serve as a catalyst for a~~l to the~~ biofuel cell. The results for glucose showed that the highest secretion overall was 0.293 mmol/L and occurred in the 2x12mm scaffold after 21 days of culture. For glucose, the p-values for culture period, scaffold size, and the interaction between the two were highly significant. This showed that both factors, as well as the interaction between the two affected the concentration secreted. For free fatty acid the highest concentration secreted was 872.733 µM, a

secretion level that occurred on the 2x10mm scaffold after 18 days of culture. The p-values for culture period and the interaction between culture period and scaffold size were found to be highly significant. Finally, the highest concentration secreted of triglyceride was found to be 1.878 (mmol/L) on the 2x10mm scaffold after seven days of culture. Again, the p-values for culture period, scaffold size, and the interaction between the two were highly significant showing that both factors, as well as the interaction between the two affected the concentration secreted. Over time, secretion increased steadily. It was found that the 2x6mm scaffolds were the most unpredictable, as the amount of secretion increased and decreased sporadically. According to the data collected the most successful scaffolds, in relation to the amount of organic compound secreted, are scaffolds size 2x10mm or 2x12mm, around day 18 or 21. This data differs from the data on glycerol. The glycerol secretion was most successful in scaffolds size 2x6mm and 2x10mm. The highest levels of error occurred in the testing for the concentration secreted of triglyceride since the concentration was found in µM and not in mmol/L. These results will help to determine what scaffold size and culture period should be used what tissue engineering adipose for the biofuel cell. It is likely that the overall study on this biofuel cell will focus on using glucose or glycerol as the main fuel source for the battery. However, the scientists researching this cell hope to incorporate all the organic compounds that can into fuel for the biofuel cell.

Conclusion

Knowing the concentrations secreted of different organic compounds when the scaffold size and culture period is changed helps to evaluate what scaffold size and culture period should be used on the eventual fuel cell. Even more accurate results could be obtained by repeating these assays multiple times at more time points. In this study the pore size on each scaffold was the same, but future studies should look into the effects of pore size on the secretion of the same three organic compounds. Additionally, future research is needed to dictate what organic compound the biofuel cell will rely most heavily on. It is according to that information that the size scaffold and cell culture period should be determined. Currently, the author of this paper is designing a construct that can contain multiple different size scaffolds for the benefit of the biofuel cell. If multiple scaffold sizes can be incorporated into the biofuel cell when using tissue-engineered adipose, the biofuel cell maywill be able to function more successfully. Future work includes designing the biofuel cell, testing the cell in vitro, testing the cell in vivo in animals, and finally testing the cell in vivo in humans.

Bibliography

Wood, M. A., & Ellenbogen, K. A. (2002). Cardiac Pacemakers From the Patient's Perspective. *Circulation, 2002*(105), 2136-2138. doi:10.1161/01.CIR.0000016183.07898.90

An Implantable Biofuel Cell for a Live Insects. Michelle Rasmussen, Roy E. Ritzmann, Irene Lee, Alan J. Pollack, and Daniel Scherson *Journal of the American Chemical Society* **2012** *134* (3), 1458-1460

Cahill, G. F. Metabolic Role of Adipose Tissue. *Trans Am Clin Climatol Assoc.*, *1962*(73), 22-29

Zebda, A., Cosnier, S., Alcaraz, J. P., Holzinger, M., Le Goff, A., Gondran, C., & Boucher, F. (2013, March 22). Single Glucose Biofuel Cells Implanted in Rats Power Electronic Devices. *Scientific Reports*, *3*(1516), 1-5. doi:10.1038/srep01516

Horowitz, J. F. (2003, October). Fatty acid mobilization from adipose tissue during exercise. *TRENDS in Endocrinology and Metabolism*, *14*(8), 386-392. doi:10.1016/S1043-2760(03)00143-7

Cosnier, S., Le Goff, A., & Holzinger, M. (2013, September 17). Towards glucose biofuel cells implanted in human body for powering artificial organs: Review. *Elsevier*, *2014*(38), 19-23.

Wray, L. S., Rnjak-Kovacina, J., Mandal, B. B., Schmidt, D. F., Gil, E. S., & Kaplan, D. L. (2012). A silk-based scaffold platform with tunable architecture for engineering critically sized tissue constructs. *Biomaterials*, *33*(2012), 9214-9224.

Rockwood, D. N., Preda, R. C., Yucel, T., Wang, X., Lovett, M. L., & Kaplan, D. L. (2011). Materials fabrication from Bombyx mori silk fibroin. *Nature Protocols*, *6*(10).

Liu, H., Li, X., Zhou, G., Fan, H., & Fan, Y. (2011). Electrospun sulfated silk fibroin nanofibrous scaffolds for vascular tissue engineering. *Biomaterials*, *32*(2001), 3784-3793.

Anne J. Meinel, Kristopher E. Kubow, Enrico Klotzsch, Marcos Garcia-Fuentes, Michael L. Smith, Viola Vogel, Hans P. Merkle, Lorenz Meinel, Optimization strategies for electrospun silk fibroin tissue engineering scaffolds,Biomaterials, Volume 30, Issue 17, June 2009, Pages 3058-3067, ISSN 0142-9612, http://dx.doi.org/10.1016/j.biomaterials.2009.01.054. (http://www.sciencedirect.com/science/article/pii/S0142961209001185)