

Customized 3D Printing of Live Cells for Novel Bio-Circuitry

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ABSTRACT

The revolutionized biomedical industry has a deep connection with the utilization of synthetic biology, to create multi-functional, complex but minimalistic, non-invasive biomedical devices. Bioelectronics have constructed new methods to monitor activity of cells and tissues that will likely advance the medical field to monitor the human body. As a new industry, bio-circuits are faced with emerging problems, this research is focused on the inability to construct and test circuits. This research proposes the implementation of a 3D printer to accurately produce the complexity of a biosensor. The engineering goal was to print 3 dimensional algal biological circuits that would conduct electricity at a 0.4% recovery rate of the controlled circuits voltage. Petroleum based ink cartridges represented the controlled group while being compared to algal bio-ink representing the experimental group. Each ink was printed onto a transparent film sheet and tested for electrical conductivity using a multimeter. The controlled data averaged 28.16mV, 26.77mV, and 5.57mV for one layer and the experimental data averaged 0.1mV, 0.1mV, and 0.0mV for one layer. The experimental data had an average numerical increase of $y=3E-5x$. With an equalization point at 28.16mV. The algal bio-ink will need 933.33 layers creating a 2.799mm algal layer. The circuit was engineered out of algae which meant that the goal of engineering a completely biological circuit was met. The engineering goal was achieved because the experimental group was able to produce a 0.4% recovery rate as compared to the controlled circuit group.

Introduction

The revolutionized biomedical industry has a deep connection with the utilization of synthetic biology [Figure 1A], to create multi-functional, minimalistic, and non-invasive biomedical devices. These devices have created a revolution in medicine to find new ways to solve a situation with minimal invasion and have it serve many functions while it is being used [1]. Many early devices such as the first novel glucose monitor which uses principles of thermal emission spectroscopy to demonstrate glucose measurements with clinically acceptable accuracy, created a new solution by changing one variable like daily calibration[2]. These small but

important changes have produced opportunities for others to solve more recent and advanced problems like using nanoelectronic brain probes to overcome the mechanical mismatch between soft tissues and hard electronics. The most apparent contributions of biomedical engineering in medical practices involve instrumentation for diagnosis, therapy, and rehabilitation. Cell and tissue engineering also have emerged as medical realities. In the next 25 years [Figure 1B], advances in electronics, optics, materials, and decreasing the size of devices will accelerate development of more sophisticated devices for diagnosis and therapy, such as imaging and virtual surgery [Figure 1C].

A current medical advancement and an emerging method utilizes mathematically advanced optimization of biological circuits or bio-circuits[3]. Bio-electronics have created new

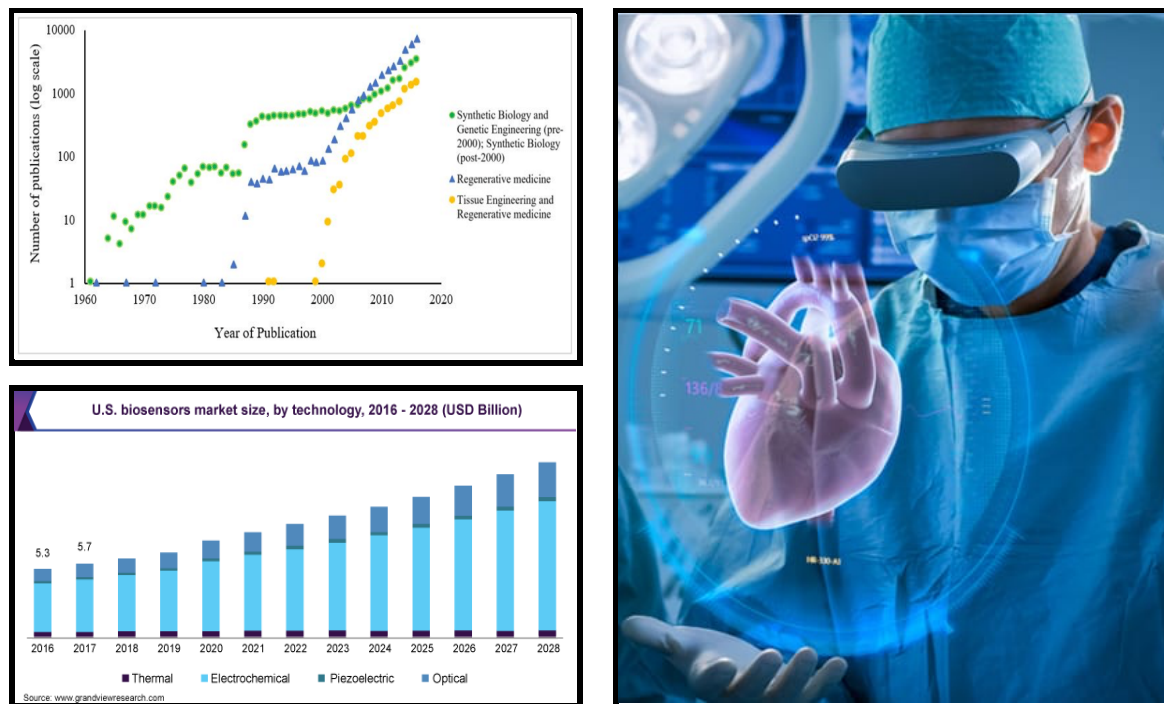
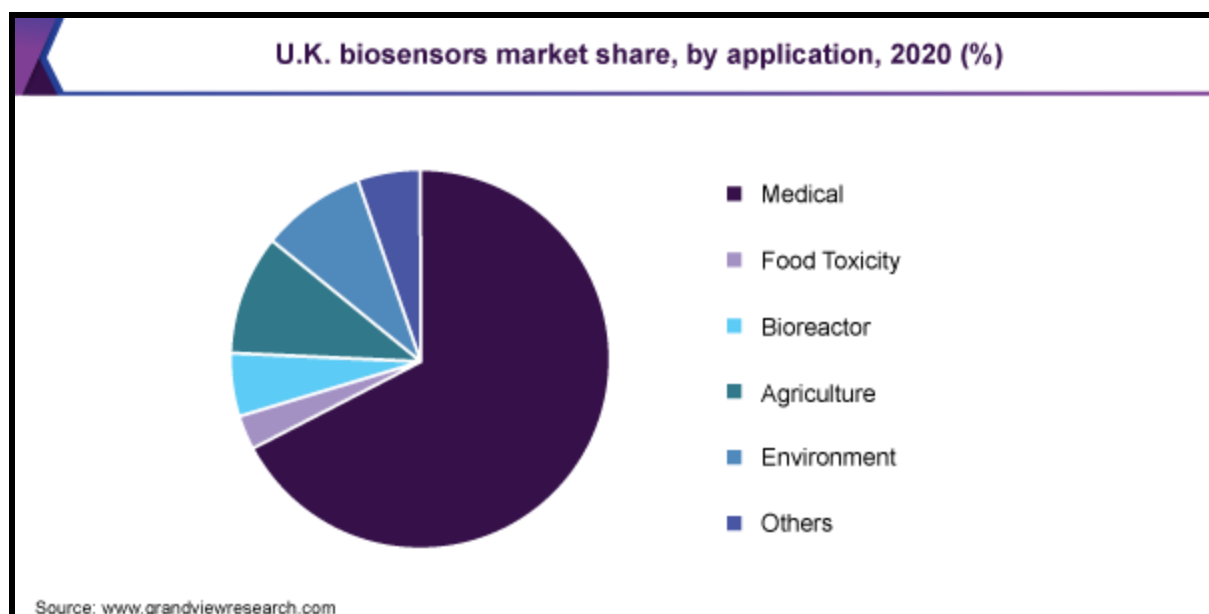


Figure 1: Top Left:[A]; analysis of publications in synthetic biology. Bottom Left: Figure[B]; technological growth of the biosensor market. Right: Figure[C]; virtual reality surgery.

ways to monitor the reproduction of cells and tissues that will likely advance the medical field to monitor bone, muscle, soft tissue, and organ growth and functionality[4]. These bio-sensors have begun a new challenge to integrate different modules and create circuits that are progressively more mathematically advanced in their programming [Figure 2]. But the problems that synthetic biologists are facing is that each biosensor is rooted with simplistic and uni-functional design flaws, whereas the medical industry needs devices that can carry out multiple functions and produce complex data analysis[5]. This problem on top of two other undisputable problems; the inability to control multiple cellular processes and increasing problems to construct and test circuits, have created a road block for scientists to advance in the bioelectronic field[6].

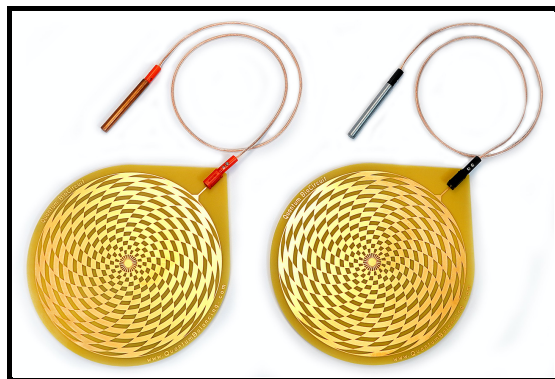
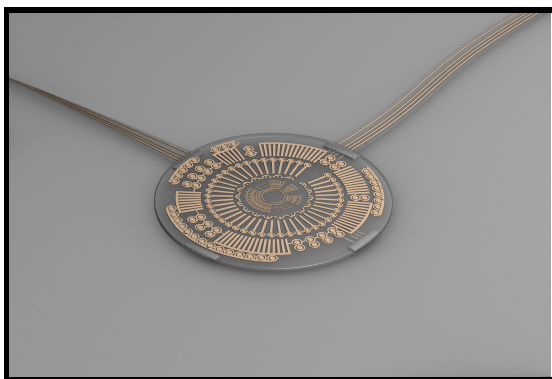
The problem investigated in this research was the inability to construct and test circuits. The construction of circuits has to be described as complex but in a simple manner; complex in the content of bio-sensor design but created in a simple and attainable procedure. This could be



Figure[2]; different subtopics of the biosensor market share

attained by using a 3D printer to accurately produce the full design of the bio-circuit. Testing the bio-circuit could be done by monitoring the voltage that was produced from the bio-sensor. Another problem that was investigated in this research was to print a biologically advanced bio-circuit as compared to prior circuits. Many prior bio-circuits were made out of hydrogels that contained biological materials like protein-polysaccharide chains that typically contain 90-99% water and a silver or copper based circuitry [Figure 3A and 3B], but an entirely biological circuit should have a living element in the circuitry like algae.

The engineering goal was to 3 dimensionally print algal bio-circuits that could conduct electricity at a 0.4% recovery rate as compared to the controlled group. The 0.4% recovery rate was used to show a statistical measurement between the controlled and experimental groups. The rate was found by dividing the average controlled group voltage by the voltage that the group was given from the power source. The controlled group had highly conductive materials (2-pyrrolidone, butane-1, 4-diol, and glycerol) and the experimental group contained non-conductive materials, (algal culture medium and algae) so the conductivity difference needed to be represented in the recovery rate, thus why the 0.4% was used as a baseline.



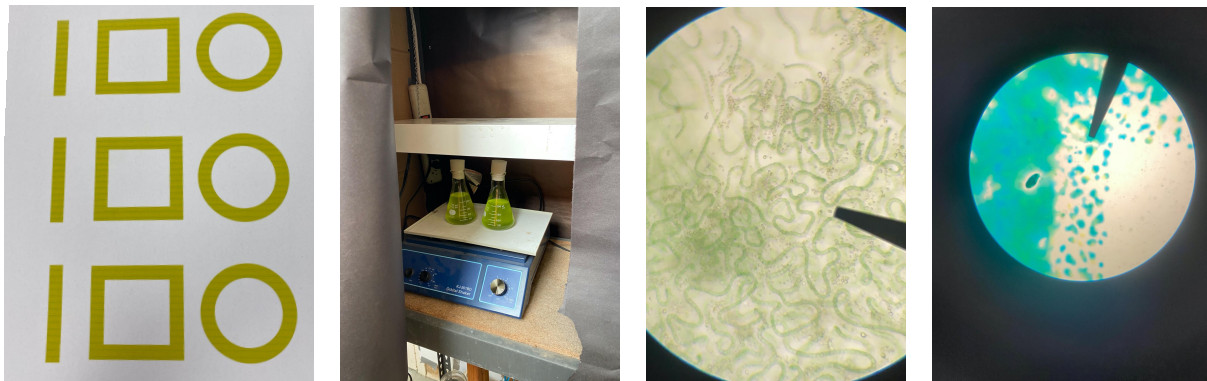
Left: Figure[3A]; biocircuit design¹² Right: Figure[3B]; biocircuit design¹³

Materials and Method

Chlorella vulgaris, a freshwater algae that was low cost and locally abundant, was cultured in 5 flasks each filled with 100 mL of Alga-Gro®: algal culture medium, that had a pH of 7.6- 7. 8. The *Chlorella vulgaris* was put onto a shaker table at 50 rpm with white fluorescent lights providing 1500 lumens/m². A timer was set for 8 hr of darkness and 16 hr of light to represent the daily cycle of natural light from the sun [Figure 4A].

Phase 1:

An ink-cartridge was modified to be able to print *Chlorella vulgaris* in the liquid growth medium. For the controlled group, a regular ink cartridge was placed in the printer and then a series of shapes were sent through the printer and printed [Figure 4B]. The experimental group was conducted by replacing the ink-filled cartridges with the algae-filled cartridge. Once all trials were completed they were microscopically analyzed with photo-recognition technology and a microscope with photography capabilities to determine the printed area of ink [Figure 4C and 4D].



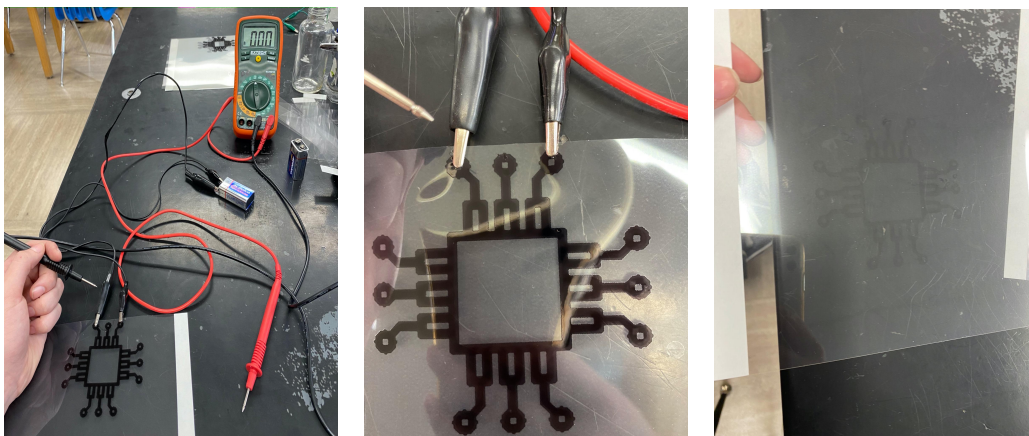
Left: Figure[4A]; shapes used to analyze the dimensions. Middle Left: Figure[4B]; Controlled environment for algae. Middle Right: Figure[4C]; 400x scale microscopic reference of algae. Right: Figure[4D]; Microscopic reference of printer ink.

Phase 2:

A second set of trials was used to determine if the printer was capable of printing a circuit out of algae. The same ink cartridge was filled with the biological ink and was placed into the printer to demonstrate the ability. After the dimensions of both groups' shapes were equalized, another set of trials was produced to determine the amount of electrical output that both of the inks could produce. For controlled group data, the pigment based ink was placed in the printer and was printed out and hooked up to 7.6 V of direct current battery from silver alligator clips. This was tested by using a voltmeter set to 2 mV [Figure 5A]. The experimental group followed the same conditions with the exception of algae instead of pigment-based ink (n=10) [Figure 5B and 5C].

Phase 3:

After analysis of the printer dimensions, A third analysis was needed to figure out the statistical advancement of layer-by-layer printing in the experimental trials. The same process was used but



Left: Figure[5A]; circuit voltage testing set-up. Middle: Figure[5B]; controlled group. Right: Figure[5C]; experimental group.

the algae was printed out layer-by-layer on the same piece of a clear sheet for 20 trials. This analysis was needed to find equalization points between the controlled and experimental groups and to obtain theoretical measurements of the experimental group's printed ink.

Results

Phase 1:

The first phase of research revolved around analyzing the printer dimensions. After 30 trials of printing 3 different shapes, the dimensions showed a 0% deviation between the dimensions.

Phase 2:

For the singular analysis of circuit voltage testing 10 trials (N) of the controlled data set produced an average voltage of $28.16\text{V mV} \pm 3.3\text{ mV}$ for the left leg, $26.77\text{ mV} \pm 2.8\text{ mV}$ for the right leg, and $5.57\text{ mV} \pm 2\text{ mV}$ for the back leg. For the experimental group, 10 trials (N) produced an average voltage of $0.1\text{ mV} \pm 0.4\text{ mV}$ for the left leg, $0.1\text{ mV} \pm 0.03\text{ mV}$ for the right leg, and the back leg was not traceable on the electrometer that was used [Table 1 and 2].

Table 1. Demonstrating the average voltage recorded from the dye-based ink trials.

Controlled Group Data (mV) Dye-Based Ink			
	Left leg (mV)	Right leg (mV)	Back leg (mV)
Layers (n)	10	10	10
Average	28.16	26.77	5.57
SD $\pm$	3.36	2.79	2.04

Table 2. Demonstrating the average voltage recorded from the algal bio-ink trials.

Experimental Group Data (mV) Biological Ink			
	Left leg (mV)	Right leg (mV)	Back leg (mV)
Layers (n)	10	10	10
Average	0.1	0.1	0
SD±	0.42	0.03	0

Phase 3:

The secondary analysis for the layer-by-layer testing was increasing by 0.1 mV at roughly every fourth trial increment. Because of this incrementation, a more in-depth analysis and equation was needed to mimic the relationship between the increments [Table 3].

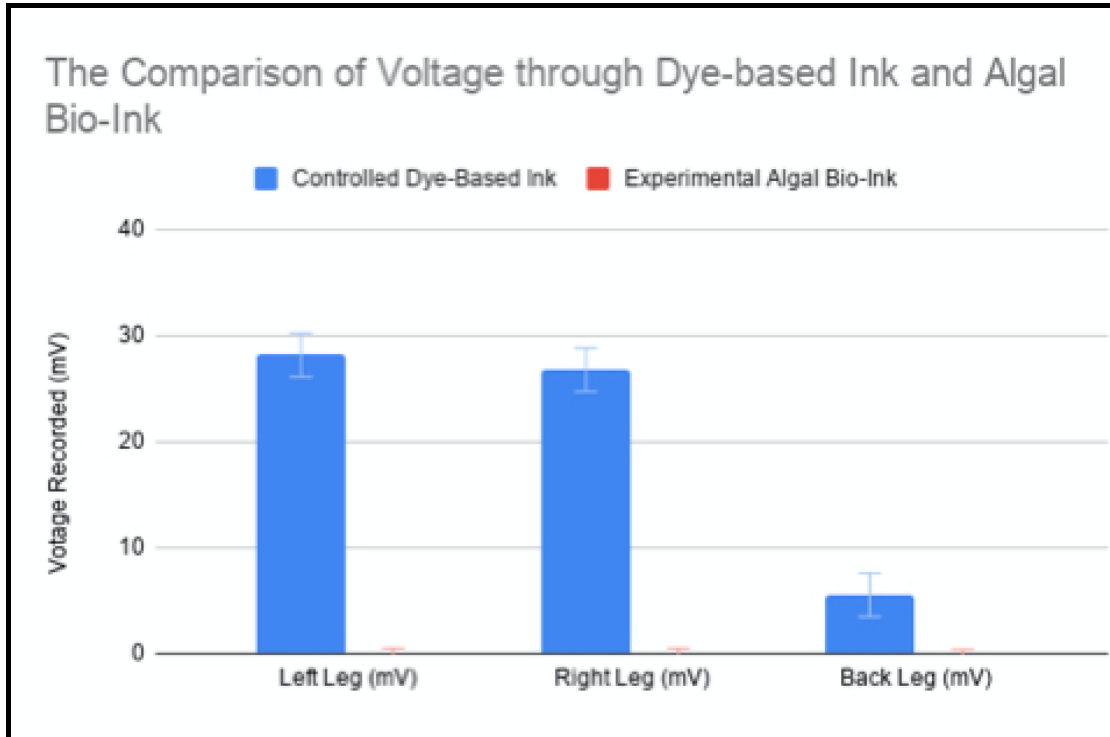
Table 3. Demonstrating the voltage increase in trial increments of 0.1mV from the biological ink.

Biological Ink Numerical Increase by Layer				
Layer	Left Leg (mV)	Right Leg (mV)	Back Leg (mV)	Battery (V)
1	0.1	0.1	0	7.6
2	0.1	0.1	0	7.6
3	0.1	0.1	0	7.6
4	0.1	0.2	0	7.6
5	0.2	0.2	0	7.6
6	0.2	0.2	0	7.5
7	0.2	0.2	0	7.5
8	0.3	0.2	0	7.5
9	0.3	0.2	0	7.5
10	0.3	0.3	0	7.5
11	0.3	0.3	0	7.5
12	0.4	0.3	0	7.5
13	0.4	0.4	0.1	7.5
14	0.4	0.4	0.1	7.5
15	0.4	0.4	0.1	7.4
16	0.4	0.4	0.1	7.4
17	0.5	0.5	0.1	7.4
18	0.5	0.5	0.1	7.4
19	0.5	0.5	0.1	7.4
20	0.5	0.5	0.1	7.4

Discussion

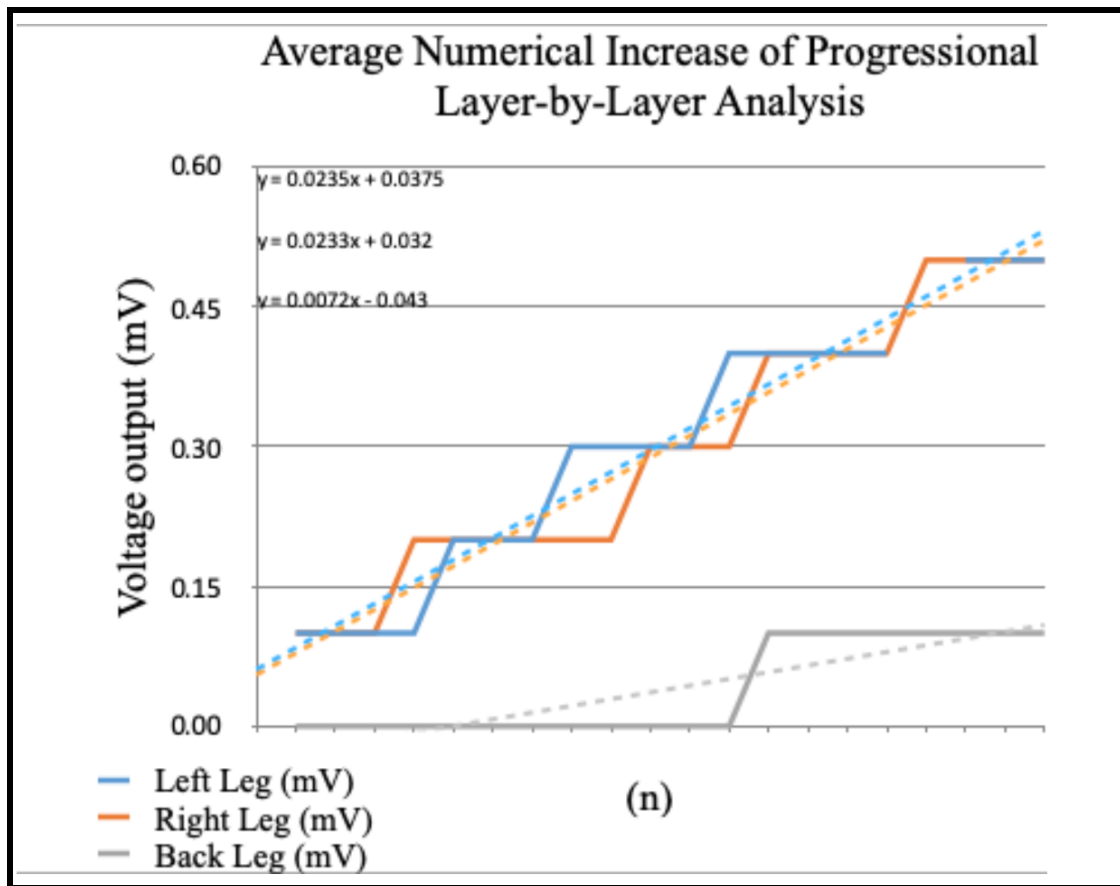
This research was designed around bio-circuits and the two issues of focus were, the inability to test and construct circuits and to construct that circuit out of only biological materials. The testing features were analyzed using an electrometer and the circuit was constructed using a customizable 3D printer. The 3D printer was able to meet specific design requirements like being customizable, cheap, low maintenance, and simple to use. The circuit was engineered out of algae which meant that the goal of engineering a completely biological circuit was met.

The engineering goal of this project was to 3-dimensionally print algal bio-circuits that could conduct electricity at a 0.4% recovery rate as compared to the controlled group. This goal was achieved because the experimental group was able to produce a 0.4% recovery rate as



Figure[7]; the comparison of voltage in circuit voltage testing graphic.

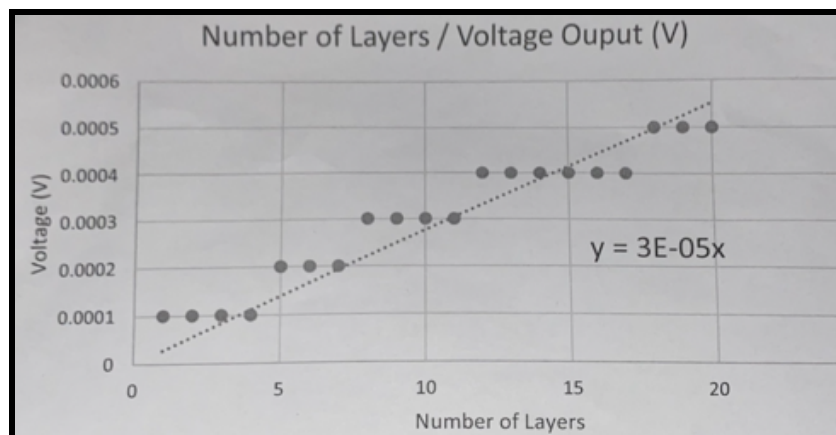
compared to the controlled circuit group [Figure 7]. After analyzing the layer-by-layer testing trials, an equation was procured to demonstrate the incrementation; the equation of $y=3E-.05X$ was used to model this relationship. This equation used the increments from the 20 trial procedure to create a theoretical pattern demonstrated in the trials. Once the equation was obtained, it was then used to determine how many layers it would take to produce a voltage equal to the controlled voltage averages [Figure 8 and 9] . The number of layers that was found was 933 layers and a relative algal size at 2.8 mm for the left leg and 892 layers and a relative algal size at 2.7 mm for the right leg. These numbers were calculated to obtain a theoretical representation of the layer-by-layer analysis.



Figure[8]; progressional reference of incrementation in the layer by layer analysis

Improvements that could be to this project include, using a stable power source, using a more dense algae, and using a more advanced voltmeter. The power source that was used was decreasing in voltage as the trials went forward and it had a 7.6 V voltage. If the circuits were allowed a stable and stronger voltage then they would be able to produce a higher voltage which could be used to make the circuit perform electrical functions. With both improvements being said, the power supply was able to pick up a traceable voltage for five of the six data points which yielded the data necessary for this research. The electrometer that was used was only able to read a voltage for the right and left leg of the experimental circuits, but it could not read the back leg. Although this was not necessary to have that number, this finding completed the data point.

Studies that are being run now consist of a growth analysis of the algae after being printed to see if the algae is living and reproducing after being printed and the size of the circuits were decreased to demonstrate a more industry standard application in the synthetic biology field. Future studies include a photosynthetic analysis to see if the algae can use their abilities of photosynthesis to obtain the energy. This would make the system more efficient and abundant. Another study is to use a hydrogel as a base to complete the biocircuits model.



Figure[9]; finalized equation calculated by taking the number of layers as it relates to voltage increase

Literature Cited and References

1. McDermott KW (IBM Watson Health), Freeman WJ, Elixhauser A (AHRQ). Overview of Operating Room Procedures During Inpatient Stays in U.S. Hospitals, 2014. HCUP Statistical Brief #233. December 2017. Agency for Healthcare Research and Quality, Rockville, MD.
2. S I Berríos-Torres, MD; C A. Umscheid, MD, MSCE². Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, JAMA Surg. 2017;152(8):784-791. doi:10.1001/jamasurg.2017.0904.
3. “Preventing Surgical Site Infections” The StayWell Company, LLC. Yardley, PA. Retrieved from: <https://www.fairview.org/patient-education/89221>.
4. Harold C: The Crisis in Antibiotic Resistance, Science August 21, 1992: Vol. 257, Issue 5073, pp. 1064-1073. DOI: 10.1126/science.257.5073.1064.
5. Solomon SL, Oliver KB. Antibiotic resistance threats in the United States: stepping back from the brink. Am Fam Physician. 2014 Jun 15;89(12):938-41. PMID: 25162160.
6. Noel SP, Courtney H, Bumgardner, J.D. et al. Chitosan Films: A Potential Local Drug Delivery System for Antibiotics. Clin Orthop Relat Res 466, 1377–1382 (2008). <https://doi.org/10.1007/s11999-008-0228-1>.
7. Takeuchi, H. Yakugaku Z: Journal of the Pharmaceutical Society of Japan vol. 140,8 (2020): 1013-1024. doi:10.1248/yakushi.20-00011.
8. Dellinger EP, Gross PA, Barrett TL, et al. Quality Standard for antimicrobial prophylaxis in surgical procedures. Clin Infect Dis 1994; 18: 422–7.
9. Plager, J; Mancini, C; Shenoy, E; Melnitchouk, S; Collier, L; et al. Journal of Allergy and Clinical Immunology; St. Louis Vol. 145, Iss. 2, PP 208.
10. Shah, Eric D MD, MBA¹; Hernandez-Barco, Yasmin G. MD²; Beck, Kendall R. MD³; Levy, Benjamin H. III MD⁴; ACG FDA-Related Matters Committee The American Journal of Gastroenterology: September 2020 - Volume 115 - Issue 9 - p 1351-1352.

11. Mio E, Ryohei H, Hiromi S, Akitomo Y, Midazolam Intoxication in a Premature Neonate
Clinical Therapeutics, Volume 42, Issue 5, 2020, pp. 946-951.
12. "Collections." *QuantumBalancing.com*, quantumbalancing.myshopify.com/products/.
13. "James Rodney - Biocircuits Concept." *Bio-Circuitry*, 2010, jrodney.com/biocircuits.