Development of a Non-invasive Cortisol Sensor

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Abstract

Cortisol is a hormone synthesized by the human body during a sympathetic nervous response and acts as a biomarker for stress. Current methods of cortisol collection and analysis consist of saliva, blood, and urine tests. Although these methods prove to be accurate, they are invasive of the testing subject and are time consuming in the analysis. In order to create a less invasive, quicker, and overall more efficient test for cortisol detection, the Colorado Space Grant Consortium Wearables Quest team has designed a piece of wearable technology that allows the user to accurately and comfortably measure their cortisol levels. The mentioned device consists of an Organic Electrochemical Transistor (OECT) and Molecularly Imprinted Polymer (MIP) itself as well as an accompanying computational device. This device is then incorporated into a wearable waistband for the user to comfortably wear in day-to-day activities. Prototyping as well as final construction of the wearable will be carried out by the Wearables Quest team as well as a graduate mentor from McLeod Lab's to complete the final piece of functional as well as efficient wearable technology.

1.0 Introduction

The purpose of this project is to design a non-invasive wearable to measure the stress levels of astronauts using cortisol. This is a resource for astronauts to better understand and treat the impact that high stress environments have on their physical and mental health. Currently, astronauts attempt to measure stress levels using heart rate monitors. While they may be more convenient than traditional methods, heart rate monitors do not provide the user with as accurate readings as measuring cortisol directly would. Therefore, this project serves as a means to measure cortisol at point of care with more accurate results.

2.0 System Overview

The wearable device is used to measure cortisol and consists of two main systems, namely, the electrical system and the structural system. These systems work in conjunction in order to measure cortisol and extrapolate data from it, which can then be used to reduce stress.

The waistband is the structure of the wearable device, which houses the electrical system. It sits along the lower back of the test subject, which allows the sensor to collect sweat from the skin without causing discomfort to the test subject or allowing the electronics to get wet. Additionally, the waistband can be easily accessed in order to retrieve data or to clean the sensors.

The electrical system has three key functions: it draws cortisol into the Organic Electrical Transistor (OECT) and Molecular Imprinted Polymer (MIP) assembly, detects changes in the current and voltage of the OECT, and stores the data collected. The foundation of the electrical system is the OECT which when used with the MIP, allows for the collection of cortisol from sweat. A microcontroller then can be used to assess the differences in voltage and current caused by the collection of cortisol, which can be used to assess the wearer's stress.

2.1 Structural Design Elements

Many factors were considered when deciding where the structure of the wearable device would be situated on the astronaut. The wearable needed to be in a place where sweat was easily accessible, that was comfortable for the individual using the device, and somewhere that was functional to allow for attaching the electronics. While the initial idea was to use the wrist to place the wearable structure, a discussion with an engineer at NASA revealed that astronauts have limited space on their arms for new devices. Therefore, the best place for the wearable device is the lower back of the individual. The lower back collects sweat easily and it is a functional and comfortable place to have a wearable device on when completing physical activities.

The structural component of the wearable is a waistband that will be positioned on the lower back of the individual. This waistband holds all of the electronic components, including the microcontroller, wires, and battery pack within a pouch. The wires are then connected through a hole in the pouch to the OECT. Apart from the waistband, the OECT and MIP are placed on the individual's lower back using medical-grade adhesive to keep it in place.

There were many elements to consider when determining the waistband structure. First, the material has to be comfortable since an individual will have to be wearing it all day. It also must be water-resistant because the individual is expected to sweat heavily. Finally, the structure needed to be durable enough to carry materials while not getting ruined during physical activities. This is especially important because the structure needs to carry electronics safely while the astronaut completes their mission. The waistband chosen was designed for running, which fit all of this criteria and was very affordable. A 3D printed electronics casing was designed to protect the electronics and ensure the durability of the device fitted in the waistband design (Figure 1).

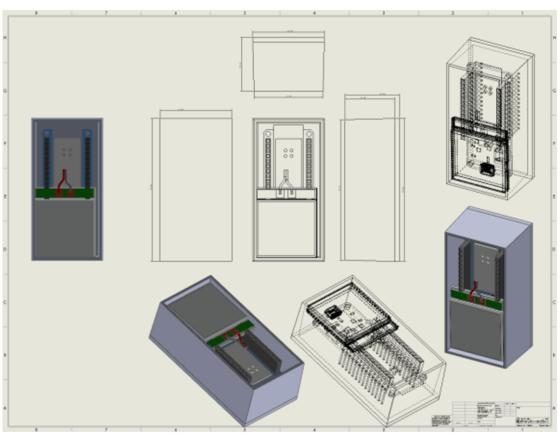

Figure 1: Electronics Casing Design

2.2 Subsystem A: The Organic Electrochemical Transistor

The cortisol sensor itself is the subsystem of the device that involves the biosensor responsible for the detection of cortisol in a sweat sample. It consists of an Organic Electrochemical Transistor (OECT) as well as a Molecularly Imprinted Polymer (MIP) to translate cortisol levels into a usable electrical current. The OECT is a biosensor that uses the injection of ions from an electrolyte to control a current between a drain and a source electrode [1]. The OECT consists of a semiconductive polymer channel, three electrodes, and an electrolyte solution. A source electrode is typically grounded, while a voltage is applied to the gate and drain electrode (Figure 2). The semiconductive polymer channel is made up of a poly(3,4-ethylenedioxythiophene) or PEDOT semiconductor which is oxidized (p-type doped) by the poly(styrene sulfonate) or PSS anions, resulting in a conductive PEDOT:PSS complex. A controlled voltage is applied to the gate, which controls the injection of cations from the electrolyte solution and into the channel. These cations replace the PSS ions, which alter the channel's doping state and make the channel less conductive [2]. The OECT acts as a depletion mode transistor [3]. When no voltage is applied to the gate electrode, the OECT is in its "ON" state, and electrons can move across the channel with relatively minimal resistance. When a voltage is applied to the gate electrode, the OECT switches to an "OFF" state, as cations are injected into the channel and reduce its conductivity [4].

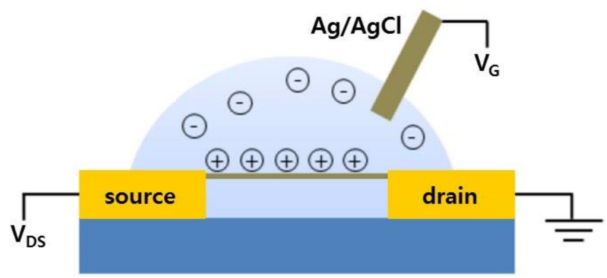

Figure 2: An Organic Electrochemical Transistor

Subsystem A uses Ag^{+}/AgCl ink as its electrodes, which are painted on a glass substrate. The electrode geometry is in a single plane, with source and drain electrodes on either side of the channel(Figure 3). In testing, solutions of artificial sweat are used as the electrolyte solution, with sodium ions in the sweat being injected into the channel.

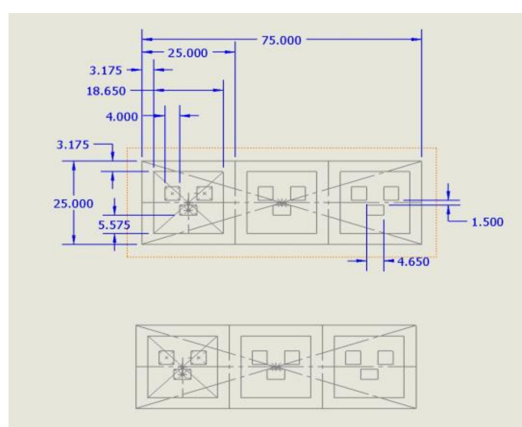

Figure 3: OECT Electrode Geometry (Measurements in Millimeters)

2.3 Subsystem B: The Molecularly Imprinted Polymer

The analyte being tested for, cortisol, is a nonpolar substance and cannot be detected by the OECT itself. The transistor can indirectly measure cortisol concentration with the use of a MIP. MIPs are sponge-like polymers that have an affinity towards a particular template molecule, or cortisol in this case. This MIP is positioned between the sweat sample and the OECT (Figure 4). As the sweat solution diffuses across the MIP and into the OECT's channel, cortisol binds to unique functional monomers in the polymer, blocking and building resistance that prevent ions from flowing through to the channel. As a result, the PEDOT:PSS complex is depleted at a slower rate, which can be measured by the transistor[1].

In this self-assembly molecular imprinting, the template molecule is combined with functional monomers that are unique to the template molecule's structure. Subsystem B used a methacrylic acid functional monomer to bind with the template molecule, cortisol. Once this solution of functional monomers binds to the template molecule, cross-linkers (ethylene glycol dimethacrylate) secure the monomers in place. The polymerization process is initiated with a photoinitiator, securing the monomers and cross linkers into the matrix of the polymer. For testing purposes, a Non-Imprinted Polymer (NIP) will be synthesized without a template. The polymer is then mechanically ground before the template molecule is flushed out of the polymer matrix using an acetic acid/methanol mixture. This final MIP is adhered to the OECT in an MSM matrix.

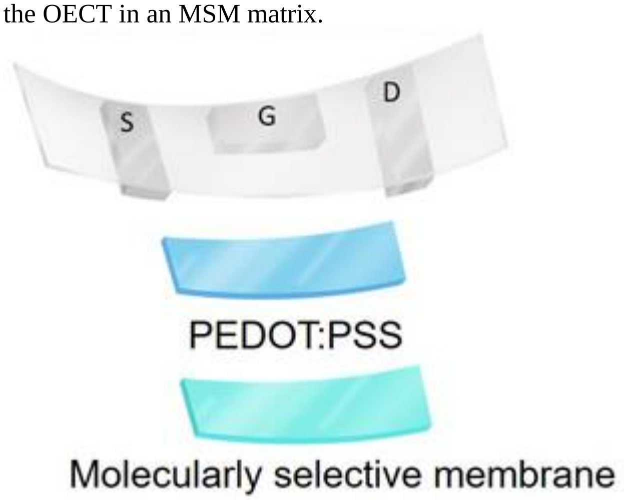

Figure 4: OECT-MIP Complex [1]

2.4 Electrical and Software Design Elements

The OECT will be connected to and powered by an Adafruit Metro 338 microcontroller. This will allow for voltages to be measured within the circuit and recorded to an SD card shield. The microcontroller will read two voltages, Vout1 and Vout2, in order to determine the current coming out of the OECTs collector, Ic. This current is directly proportional to the current coming into the base of the OECT which is caused by the ions in the sweat. By measuring Ic, the number of ions allowed to pass through the MIP into the OECT can be determined and related to the concentration of cortisol blocking the ions. The integrated circuit, TPS60400, is a DC voltage inverter used to change the positive 5 volt output of the microcontroller into a -5 volt output. This is required due to the positive nature of the Na+ and K+ ions in the sweat. The Metro cannot output or read negative voltages so the TPS60400 is used to invert the voltages before and after the microcontroller (Figure 5).

After reading the voltages Vout1 and Vout2, the current Ic can be determined by using the voltage dropped across the resistor, Vout2-Vout1, and the resistor's value, 5000 kOhms. This current is proportional to the number of ions in the sweat coming into the OECT by a factor of beta. Using multiple solutions of known cortisol and sweat, a model could be created to relate the current, Ic, to the amount of cortisol in the sweat. After creating the model, an unknown solution of cortisol and sweat could be tested and using the collector current, the amount of cortisol could be determined by comparing that result to the known results recorded earlier.

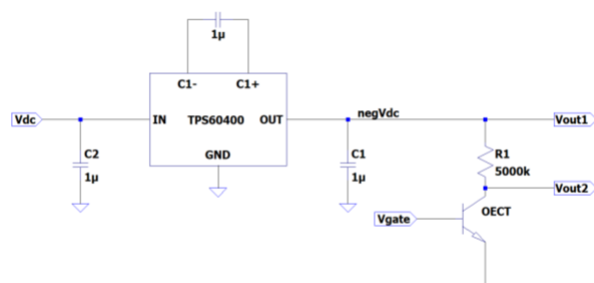

Figure 5: Circuit Diagram

This circuit diagram (Figure 5) depicts how the OECT will be integrated into a larger circuit to analyze the behavior of the OECT. Vout1 and Vount2 are inputs into the microcontroller to determine the current coming out of the transistor. The TPS60400 is an integrated circuit used to invert the positive voltage output by the microcontroller.

2.5 Prototyping and Analysis Results

After completing and analyzing the design elements of the sensor, prototyping began. This stage included the fabrication of 12 OECTs with several variables in the construction process. The 12 OECTs were first split up into two main categories; Ag^+/AgCl paste electrode devices and six evaporated gold electrode devices. From there, two Ag^+/AgCl paste devices as well as two gold devices were polymerized with the rational design, another two of each were polymerized with the adjusted design, and one of each were polymerized without the template molecule. The last one of each device was set aside to not be polymerized and used as a baseline for testing. From the eight devices that were either polymerized with the rational or adjusted design, all received the MIP. The two devices that were polymerized without the template module received the NIP and the two non-polymerized devices did not receive either imprinted polymers.

In the first attempt to follow the prototyping plan, all OECTs were broken during the manufacturing process. To save time in the second attempt, only the Ag^+/AgCl electrode devices were made. The plan had been altered to only omit the gold electrode devices, but continue as planned with the six Ag^+/AgCl electrode devices.

As the next steps in the process, the six Ag^+/AgCl devices will then be tested and analyzed for their ability to detect cortisol levels. Each unique OECT would then be analyzed in order to determine their capabilities.

3.0 Testing Plan

The overall testing plan involves testing each subsystem cumulatively, as more of the device is assembled. It should be noted that all testing will be conducted in the future, and has yet to be completed. Testing will begin with subsystem A first, and will involve characterizing the OECT first, followed by the testing of Subsystems A and B involving an OECT-MIP complex. The structural elements of Subsystem C would be tested before combining it with the two other subsystems, where testing involving the electrical and software components could be conducted.

3.1 Systems Level Testing

Subsystems A and B would have undergone two basic characterization tests. Preliminary testing would determine the resistance and conductivity of the OECT channel using a multimeter to determine viable devices. In the first characterization test, a “naked” OECT without an imprinted polymer would be injected with electrolytes and a voltage would be applied to the gate electrode at an increasing voltage. The drain current would be measured at each gate voltage and used to plot a characteristic transconductance curve. This test would be repeated on an OECT device coated with an MSM matrix to determine the resistance of the MSM to the electrolytes being injected into the OECT channel. An optimal gate voltage (V_G) would be determined and used on all subsequent tests. The next characterization test compares the Non-Imprinted Polymers (NIPs) to the MIPs. In situ, an OECT-MIP complex would be injected with an electrolyte solution mixed with cortisol in concentrations ranging from 0.01 mM to 1.0 mM. Using the difference in current between the source and the drain (ΔI_D), a calibration curve can be determined to accurately identify cortisol concentrations in any sample. These final calibration curves would be used for the final ex situ testing. For final ex situ testing, artificial sweat solutions with cortisol would be sprayed on a test subject’s abdomen, measured with the assembled wearable device, and the readings would be compared to the actual cortisol levels in the artificial sweat.

3.2 Structural Testing

The main requirements of the structure of the device are comfort and durability. As the wearable device is meant to be worn for long periods of time, it needs to be comfortable and to allow the collection of data without changing the test subject’s behavior. Additionally, it needs to be able to withstand being exposed to water and material fatigue from use.

To test the structure of the device, different conditions will be applied to the structure with the electrical system inside and attached to the test subject. These conditions include strain along the length of the structure, crushing, wet environments, warm environments, vibration, and overall daily use, in order to test the longevity and ensure protection of the sensor and electrical components. Testing will consist of test subjects simply wearing the device for extended amounts of time doing different activities including exercise and rest. This can be compared to other articles of clothing that are known for their comfort level. For example, a sweatshirt could be the most comfortable whereas a tight t-shirt could be the least comfortable. The quality of fabric can also be graded based on comparison to other fabrics such as canvas, which is seen as a low-quality fabric, versus silk, which is seen as a high-quality fabric. Breathability, flexibility, and mobility (ie, does it stay in place on the user) are other metrics that can be measured.

3.3 Software and Electronics Testing

The only requirement for the electronics system is that as cortisol changes within a given solution, so does the current measured. As long as the current changes in a predictable and consistent behavior, the value of cortisol will be able to be measured. Preferably the change in current with respect to the amount of cortisol would be constant allowing for a linear fit and simpler modeling; however, in most transistors this behavior only happens before saturation. After the OECT has been tested and modeled, the collector voltage can be varied to allow the transistor to be in the active state with common cortisol levels instead of saturation to allow for more accurate measurements.

Once the amount of cortisol has been modeled with a relationship to the current, the measured cortisol is expected to be with a range of 10% of the known cortisol within a sample.

3.4 Data Analysis

The hypothesis for this study is that there will be no significant difference between the known cortisol level in the test sample and the measurement yielded by the Organic Electrochemical Transistor (OECT). Success for the device would mean that the results measured by the transistor are statistically significant in accuracy when measured against the known levels of cortisol in the sample. Since multiple devices were fabricated, they will each have slightly different responses to the same test sample of synthetic sweat. An OECT that was fabricated under the most optimal conditions will be used to establish a calibration curve. The remaining devices will be compared to the calibration curve to correct for this systematic error in fabrication. Comparing the devices in this way allows for the creation of a confidence interval in which the true concentration of cortisol in the sample will fall. The microcontroller will then be programmed to match the current from the OECT with the corresponding cortisol concentration within the confidence interval. A Pearson's Product-Moment Correlation test will be run for each device to determine the probability of getting the result when the null is true, and if the p-value was less than 0.05 then the device would be considered successful.

4.0 Future Directions

As for the future of this project, lab operations are hoped to be resumed this summer to test the Organic Electrochemical Transistors with the synthetic sweat samples with known cortisol levels. Future directions for the project may include using OECTs to test for hormones found in sweat other than cortisol. Testing for other hormones will allow for higher confidence in the sensitivity of the devices by eliminating confounding hormones that could get caught in the Molecularly Imprinted Polymer and skew the device's reading of cortisol. Future Directions may also include expanding on the wearability aspect of the OECTs by creating devices on substrates more flexible than the current glass substrate. Once the cortisol sensor is tested and yields a significantly accurate measurement of cortisol, the power source, microcontroller, and waistband will all be connected with the sensor to complete the prototype of the wearable. Once this is complete, testing the wearable will ensure the systems all function together as a cohesive whole.

5.0 Conclusion

Stress is a fundamental and yet often underappreciated aspect to workplace environments. Aside from being detrimental to workplace productivity, prolonged periods working under high amounts of stress is detrimental to an employee's health. A better understanding of stress levels can help navigate the crucial balance between producing high-quality work and encouraging a healthy work environment. Nowhere is this more important than in high-stress and remote areas where workers often are expected to monitor their own health while still performing difficult tasks in an unfriendly or alien environment. One such example are the astronauts stationed on the ISS, who maintain and conduct research experiments in the remote and hostile environment of space. To help astronauts and other workers manage their stress, the hormone cortisol can be measured as a metric of how stressed a person is. Previous ways of measuring cortisol are invasive and require large amounts of time in order for accurate analysis. This project offers a solution that is non-invasive and time efficient; a wearable device that measures the cortisol levels in an astronaut's sweat. By making the device comfortable and non-invasive, the astronaut is able to conduct normal activities even as measurements are being taken that can be compared with a baseline to detect abnormalities in cortisol levels. The measurements taken can also be used in other research being conducted on the ISS, allowing scientists and doctors to better understand how humans adapt and behave in the alien environment of space. Accurately identifying heightened stress levels enables employers and their employees to address issues stemming from stress (ie, substandard quality of production) and improving the quality of life for those operating in those high-stress situations.

6.0 References

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7.0 Acknowledgements

Thanks to the support and guidance of McLeod labs and Megan Renny, twelve OECTS (six Ag^+/AgCl and six gold), eight MIPS (four rationally designed and four adjusted rational design), and two NIPS (polymer without template molecule) were manufactured for testing. Teaming up with the lab allowed for greater efficiency, accuracy, and problem-solving, as the expertise on-hand had greater chemistry and chemical engineering experience as well as fully equipped labs for the project to utilize.

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