The Happymeter: A Microneedle Array for Serotonin Monitoring

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Group F

Abstract

The proposed device is a point of care, minimally invasive microneedle sensor array that detects serotonin. Currently, depression and mental health in general is diagnosed by verbal descriptions of symptoms and how the patient feels. There is a thorough system for this, however, diagnosis and these verbal accounts can often be subjective rather than objective. This device would address this issue by also providing quantitative data that would let you know whether or not your serotonin levels are within the normal range. A holistic approach using both this sensor and current methods of mental health diagnosis would likely lead to much more accurate results as well as treatment plans.

Introduction

The COVID-19 pandemic has created an unprecedented mental health crisis. According to the KFF Health Tracking Poll from July 2020, there have been impacts on mental health that range from difficulty sleeping to substance use and chronic stress conditions. Much of this is also associated with the effects of job loss and isolation. Job loss as a result of the pandemic increased the rate of mental illness symptoms from 32% to 53%. Our device aims to solve this unprecedented issue by monitoring serotonin levels. Although there are research articles that have been published with various techniques that are involved with serotonin detection, a fully-fledged device that combines these functionalities as a POC device that works does not yet exist.

Although there is debate for whether one's mental state can be determined by measuring their serotonin levels, it is evident that serotonin plays an important role in nervous system diseases such as depression, obsessive-compulsive disorder, post-traumatic stress disorder, autism, and Parkinson's disease among others.[1,2] In addition, extracellular serotonin in skin is a byproduct of the body's inflammatory processes and yields more information about the body's neuroendocrine system and skin. Skin cells such as melanocytes have been shown to produce serotonin and regulate the immune and vascular systems in the skin.[3] Therefore, in addition to being an important neurotransmitter for the conscious mental states, it is also an important molecule that regulates many critical processes the body performs unconsciously.[4]

Due to the role serotonin plays in the human body, we need highly sensitive and low cost detection methods to understand the role of serotonin not only for research purposes but also for point of care measurements for people to monitor both their conscious and unconscious mental state.

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[1] No evidence that depression is caused by low serotonin levels, finds comprehensive review

[2] New insights into how serotonin regulates behavior

[3] The skin as a mirror of the soul: exploring the possible roles of serotonin

[4] Serotonin and consciousness – A reappraisal

Serotonin is found in large concentrations primarily in the bloodstream, and some of it seeps through the skin and can be found in the ISF at much lower concentrations. Serotonin in the brain is what regulates your mood and is colloquially referred to as the "feel good" chemical. Serotonin at normal levels means you will be more focused, emotionally stable, and overall happier. On the flip side, lower serotonin levels are associated with depression. Either way, when serotonin levels are too high or too low, this has the potential to create both physiological and psychological health issues.

Serotonin (5-hydroxytryptamine or 5-HT) is important for the mediation of interactions between the neuroendocrine system and the skin. This is where understanding its role and relationship with the skin and ISF comes into play. In the brain and the bloodstream, this neurotransmitter is distributed throughout the entire body and has important roles in the regulation of anxiety, stress response, sleep, sexual desire, and even memory. When the levels of 5-HT, the biomarker for serotonin, are altered, then the extracellular fluids have the potential to change metabolism, migration, and mitosis of target cells in the brain as well as the skin. One factor of note that influences the 5-HT system are cytokines. It was found that many cancer patients with cytokines were associated with low serotonin levels and depression. Pro-inflammatory cytokines also altered metabolism, releasing 5-HT in the CNS by regulating neuronal 5-HT activity.

Serotonin can be produced both in the skin and the gut. Human skin is known as a serotonergic system that can produce serotonin on its own. This conclusion was initiated with the discovery of the tryptophan hydroxylase enzyme in the skin which is responsible for the initial synthesis of serotonin. Additionally, both serotonin and serotonin transporters can be found in the epidermis. It's also possible that sunshine directly stimulates production of serotonin through the skin and there's research that supports the idea of this serotonergic machinery, however this data is not yet completely conclusive. As for the gut, peripheral serotonin is produced in the digestive tract via enterochromaffin cells. In fact, 95% of serotonin is created in the intestine where it is involved with hormonal and endocrine functions. Typical serotonin levels in human blood are within the 50 to 200 ng/mL range (0.28 to 1.14 micromolar/L).

There are various drugs and substances which have the propensity to decrease overall serotonin levels. These include: caffeine, nicotine, alcohol, and antidepressants. Hormonal changes can also cause lower levels of serotonin and neurotransmitter imbalances which can typically happen during puberty or pregnancy.

Currently, the conventional methods to detect serotonin are fluorescence, high performance liquid chromatography-chemiluminescence (HPLC-CL), liquid chromatography-mass spectrometry (LC-MS), capillary electro- phoresis (CE), enzyme immunoassay, ELISA and molecularly imprinted polymers (MIPs),but they lack the ability to be packaged in a small device for point of care applications.[5,6,7]Aptamers are also a promising solution to increase the

[5] A fast, high-affinity fluorescent serotonin biosensor engineered from a tick lipocalin
[6] A fluorescence turn-on biosensor based on transferrin encapsulated gold nanoclusters for 5-hydroxytryptamine detection
[7] Determination of monoamine neurotransmitters and metabolites by high-performance liquid chromatography based on Ag(III) complex chemiluminescence detection

selectivity of serotonin, but they are expensive $300 per gram and won't be able to be used for continuous sensing.[8] Although methods to detect serotonin have been extensively developed for *in vitro* studies with electrochemical methods and *in vivo* for animals, no integrated point of care devices have been developed. A point of care (POC) device must be low cost, operable at home by any patient without much training, accessible to the public, pain-free, and have rapid measurement.

Serotonin is typically not an easily accessible biomarker since 95% of the total body serotonin is found in the gut and a majority of the remaining 5% is found in the brain.[9] This leaves very little to be accessed noninvasively from other parts of the body. To develop a continuous point of care serotonin biosensor, we optimize its sensitivity through a proprietary electrochemical technique: N-shaped fast scan cyclic square-wave voltammetry (N-FSCSWV) and a novel electrode surface enhancement technique using a glass carbon electrode modified with overoxidized polypyrrole and gold nanoclusters.

Results & Discussions

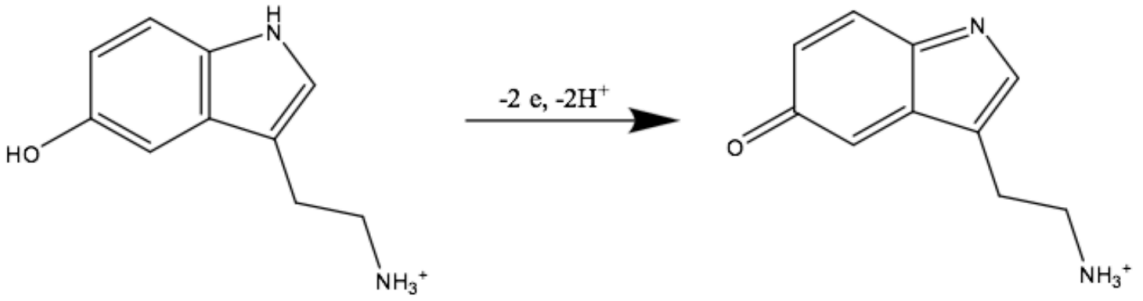

Figure 1. *Microfluidic separation and electrochemical detection of serotonin using a portable lab-on-a-chip device*

Electrochemical Detection Technique

Since serotonin is a redox active neurotransmitter, it participates in a multi-step two-electron, two-proton transfer process and therefore concentration can be measured using typical electrochemical methods such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Although those electrochemical methods are typically sufficient for detection of serotonin *in vitro,* the presence of other biomolecules such as dopamine, melatonin, ascorbic acid, glucose, and NaCl that oxidize at near the potential window of serotonin.[10] In addition, insulating films tend to form on the electrode surface at slower scan rates.[11] Therefore, in order

[8] Aptamers as targeted therapeutics: current potential and challenges
[9] The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health
[10] Nanomaterial based electrochemical sensing of the biomarker serotonin: a comprehensive review
[11] Fast-Scan Cyclic Voltammetry of 5-Hydroxytryptamine

for our point of care device to perform reliably, we chose to use an N-shaped fast scan cyclic square-wave voltammetry (N-FCSWV) technique that has been tested *in vivo* in the medial forebrain bundle (MFB) of the rat[12].

N-shaped fast cyclic square-wave voltammetry (N-FCSWV) is developed from a combination of two electrochemical techniques: N-shaped fast scan cyclic voltammetry (N-FSCV) and fast cyclic square-wave voltammetry (FCSWV).

Previously, N-shaped fast scan cyclic voltammetry (N-FSCV) has been developed to differentiate serotonin from other catecholamine neurotransmitters such as dopamine. The waveform starts at a 0.2 V holding potential and increases up to 1.0 V and back to −0.1 V and then back to 0.2 V at a scan rate of 1000 V/s. This waveform can recognize serotonin from other confounding factors by the differences in their adsorption properties.

Fast cyclic square-wave voltammetry (FCSWV) was originally developed for the detection of dopamine. A relatively large-amplitude cyclic square waveform is applied, leading to a series of oxidation and reduction within a single scan and results in a higher sensitivity of dopamine.

N-FSCWV superimposes a large amplitude square-shaped potential onto an N-shaped waveform to increase selectivity of serotonin. Contrary to typical electrochemical techniques, analytes that are potentially problematic for interference such as dopamine and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) do not significantly contribute to the serotonin signal.

[12] Sensitive and Selective Measurement of Serotonin in Vivo Using Fast Cyclic Square-Wave Voltammetry

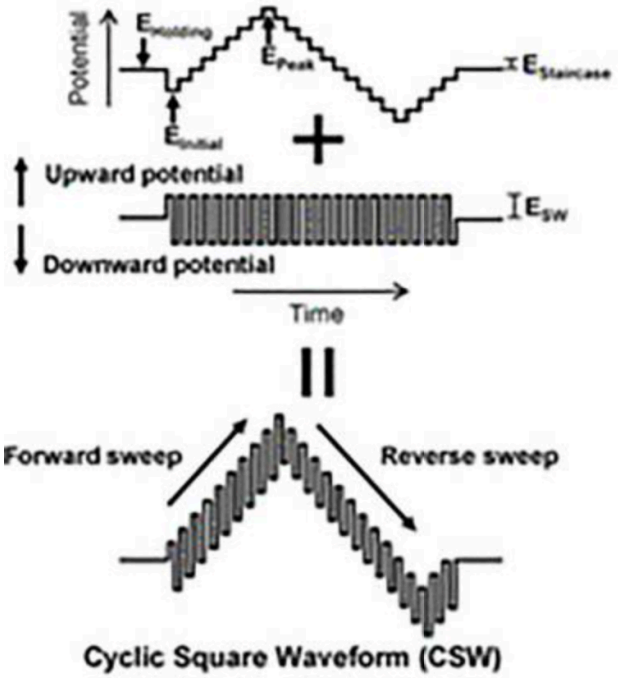

Figure 2. *Demonstrating cyclic square waveform (CSW) with the upward potential, downward potential, forward sweep, and reverse sweep components.*

Large up and down potentials can be applied in a cycle square wave to induce the oxidation and reduction of serotonin repetitively at a 5Hz repetition rate and at a 1 MHz sampling rate for the current response. The optimal parameters for potential were:

Parameter	Value
E_staircase	0.05 V
E_SW	0.4 V
E_initial	-0.1 V
E_holding	0.2 V
E_Peak	0.7 V

Having a sensitivity of 50 nM to 500nM, a detection limit of 2 ± 1 nM and a linearity of R2 = 0.9906 , the performance of N-FCSWV is better than conventional electrochemical methods with solely cyclic voltammetry and square-wave voltammetry.

Sensor Design:

To continuously detect low concentrations of extracellular serotonin in interstitial fluid, our electrodes must be modified for maximum sensitivity. Polypyrrole (PPy) is a conducting

polymer that can be overoxidized at high positive potential, suitable for electrochemical scans with N-FCSWV. Overoxidized-polypyrrole (PPyox) expels the doping ions and loses its conductivity, resulting in good insulating and molecular sieve properties.[13] In addition, PPyox is nano-porous and increases the effective surface area of the sensor. Gold nanoparticles can be deposited to further facilitate electron transfer efficacy and improve sensitivity.[14] The nano-Au/PPyox/GCE system creates a synergic effect for enhancing the serotonin current response by increasing the effective surface area and electronic conductivity.

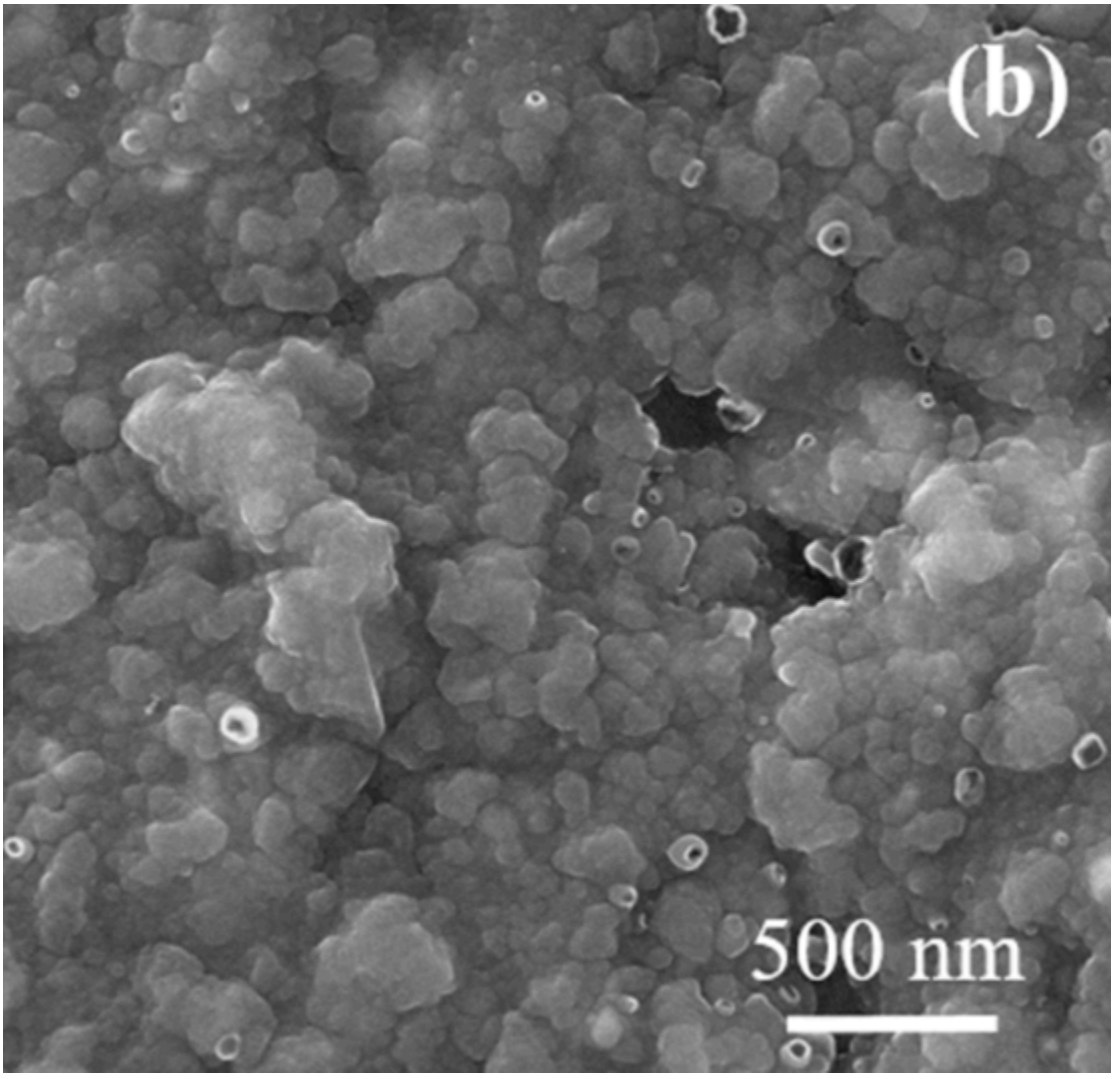

Figure 3. *Interfacial characterization of Polypyrrole/AuNP composites towards electrocatalysis of absorbic acid oxidation.*

[13] Simultaneous determination of dopamine and serotonin on gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode

[14] Interfacial Characterization of Polypyrrole/AuNP Composites towards Electrocatalysis of Ascorbic Acid Oxidation

Sensor Fabrication:

We propose the microneedles to be fabricated using a medical-grade liquid crystal polymer due to its mechanical properties (stiffness, tensile strength, resistance to plastic deformation). A suitable blend has a Young's modulus of 2350 MPa, a flexural modulus of 2300 MPa, and a hardness of 118 on the Rockwell scale. A microneedle array can be injection molded using a high-carbon stainless steel mold. A hollow microneedle tip is needed for the pass through and press-fitting of a platinum wire for the working and counter electrodes and a silver wire for the reference electrodes. A suitable geometry tested for sensing in interstitial fluid has a tetrahedral base with edges of 400 um, 800 um height, and an aperture of 100 um is optimal.[15]

To increase the surface adhesion of the polymeric microneedle array, a 400 A layer of chromium is deposited using electron beam evaporation. The glassy carbon surface is produced on the microneedle surface based on the molding of a polymer using elastomeric polydimethylsiloxane (PDMS) followed by carbonization under high pressure in an argon filled chamber.[16] This soft lithography process produces a microneedle array coated with a glass carbon surface.

Overoxidized pyrrole can be produced performing cyclic voltammetry in a dodecylsulfate sodium (SDS) and pyrrole solution by cycling from −0.35 to 0.85 V at a scan rate of 20 mV s−1 for 3 cycles. Oxidation can be performed in NaOH solution at +1.0 V (versus SCE) for several minutes until the current decreases to 8 microA.

Gold nanoparticles can be deposited using cyclic voltammetry scanning between 0.2 and −1.0 V
in a HAuCl4 solution at a scan rate of 50mVs−1 for 15 cycles.

Sensor characterization

The sensor can be characterized visually using Field-Effect Scanning Electron Microscope at all stages of the fabrication process. The surface enhancement can be quantitatively measured using cyclic voltammetry and differential pulse voltammetry salt ferricyanide salt solutions and phosphate buffer saline doped with serotonin. Key parameters to be tested include: serotonin concentrations for linearity, pH levels, selectivity against dopamine, glucose, and ascorbic acid, and inserted shelf life. Finally, testing should be performed in human blood serum before *in vivo* measurements.

[15] Continuous minimally-invasive alcohol monitoring using microneedle sensor arrays
[16] Fabrication of glassy carbon microstructures by soft lithography

POC device integration:

To accommodate the fast scan cyclic voltammetry at scan rates of 1000 V/s, a high performance modular chip is needed for a point of care device. Luckily, a few chips have been developed using the ATxmega128A3 microcontroller, similar to that of an Arduino. The chip has been tested to produce the similar Fast-Scan Cyclic Voltammogram waveforms.[17]

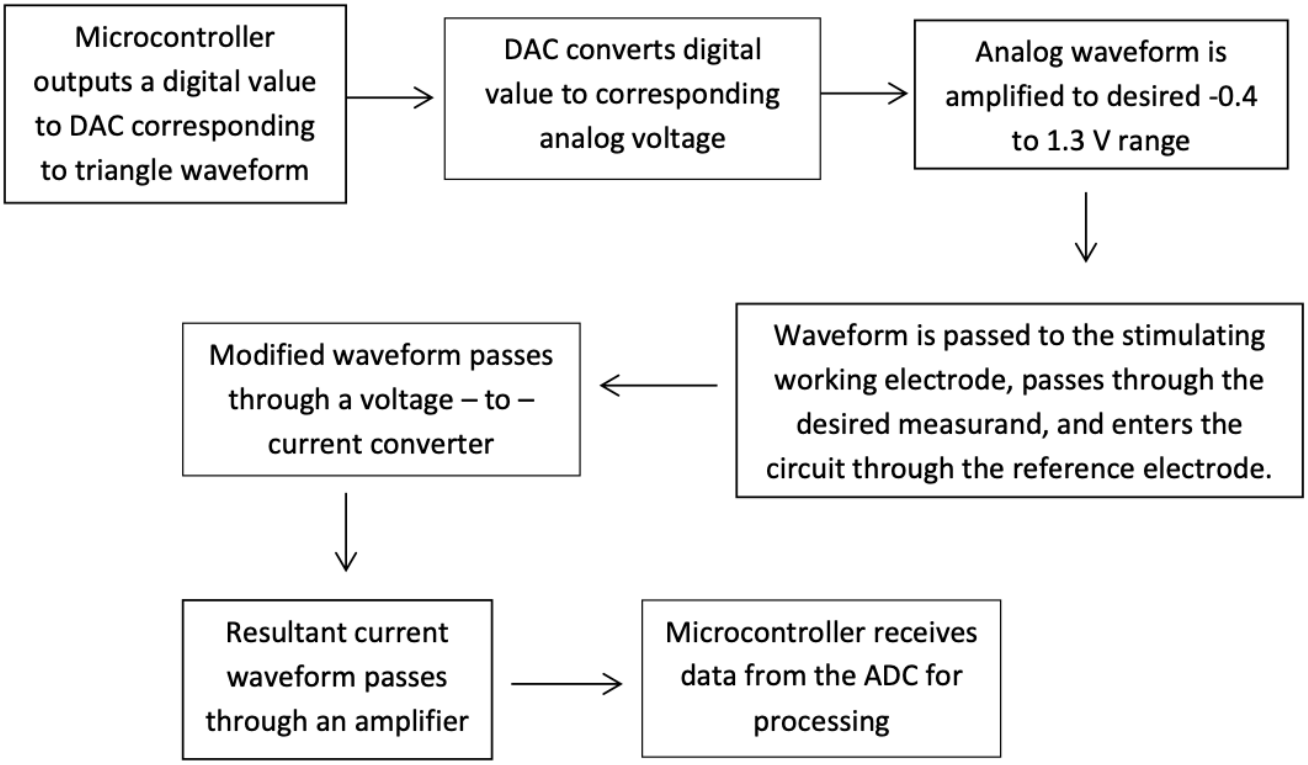

Figure 4. *Block diagram for software that operates the board (generates stimulus waveform, recording values from ADC, etc.)*

Potential problems:

Potential challenges that may arise stems from the interference of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) which is present in areas where serotonin is also present. Since 5-HIAA is an anion at the physiological pH level, a cation exchange polymer such as carbon fiber modified Nafion may be necessitated to exclude 5-HIAA from the electrode surface.[18]

[17] Design of a Portable Fast Scan Cyclic Voltammetry System for Measuring Neurotransmitter Levels
[18] Voltammetric Detection of 5-Hydroxytryptamine Release in the Rat Brain

In addition, it has been reported that an insoluble film tends to form due to the oxidation of serotonin that has insulating effects blocking the electrode and the porous in polymers typically used for the neurotransmitter detection.[19] However, these films have not been tested for electrochemical techniques using FSCV. In the event that the film forms on the electrode surface, leaving the electrode at open circuit potential for a short period of time recovers the signal.

Summary

The novel idea in this work is combining the typical methods of cyclic voltammetry and differential pulse voltammetry in this serotonin detector. The microneedles for the sensor would be constructed with medical-grade liquid crystal polymer. With the rise of mental health issues and associated disorders as a result of widespread isolation induced by the pandemic, this point-of-care device would offer a reliable way to self-diagnose mental health. Mental health has been stigmatized for decades from the general public's perspective. The device has the potential to revolutionize how mental health is regarded by legitimizing its significance with numerical evidence of it in the form of serotonin levels. Mass amounts of useful data on the mental health of entire populations would also become readily available for scientists, researchers, and public health experts alike.

[19] Investigation of film formation properties during electrochemical oxidation of serotonin (5-HT) at polycrystalline boron doped diamond

Contributions

Max Miesen: Background research, abstract, and introduction, conclusion

Max Li: Background research, manufacturing and testing portions