Correlation Between Blood and Sweat Glucose Levels for a Non-Invasive Testing Technique for Diabetics

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INTRODUCTION

- Diabetes is one of the most prevalent chronic diseases; causes uncontrollable blood glucose levels (1)
- Affects over 30 million Americans (2)
  - About 425 million adults worldwide (3)
  - Expected to increase to 629 million by 2045 (3)
- Normal fasted blood sugar level (non diabetic):
  - Less than 100 milligrams/deciliter (mg/dL) (4)
- Many techniques to monitor glucose levels exist, including nontraditional methods involving dogs detecting changes based on a chemical smell (5)
- Most people would rather utilize self-testing devices (6)
  - Worldwide market for self-monitoring of blood glucose is $2.7 billion per year (7)
  - Most common: use of blood from finger pricks for analysis in a glucometer, although this is invasive, time consuming and costly (Figure 1)

Figure 1. Glucometer testing blood glucose levels (consumerreports.org)

- Alternative technique: continuous glucose monitoring system to find trends, although this is more complicated, expensive, inconvenient and less accurate (6) (Figure 2)
- Wearable devices are on the rise; such as a tattoo-based monitoring system (8) (Figure 3)
- Demanding market in need of newly advancing, less-invasive techniques, most effective for the population

Figure 2. Continuous glucose monitor patch with a needle inserting into the arm. Tracks changes in blood sugar (time.com).

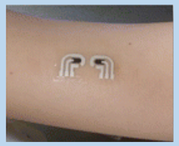

Figure 3. Tattoo-based monitoring sensor (Bandodkar, 2014).

- Utilizing a non-invasive body fluid such as sweat would make this process much more tolerable for diabetics
- Sweat is found on the skin's surface and wouldn't require invasion of needles for collection or biohazardous waste containers
- Sweat analysis techniques have been reliable in the clinical diagnosis of cystic fibrosis based on chloride levels (9)
- Sweat has recently been used to determine ethanol concentrations in drunk driving cases (Hair, 2019)

PURPOSE

The purpose of this study was to explore a less invasive method for diabetics to test their glucose levels based on the correlation between blood and sweat glucose levels.

MATERIALS AND METHODS

Enzymatic Assay Components

- Glucose oxidase (GOx) (Figure 4)
  - Enzyme highly specific for glucose; won't react with blood saccharides
- 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)
- Horseradish peroxidase (HRP) (Figure 5)
  - Catalyzes the oxidation of ABTS resulting in water
- Sodium Acetate Buffer

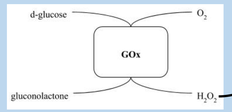

Figure 4. GOx catalyzes the oxidation of d-glucose into gluconolactone, producing hydrogen peroxide (St. Denis, 2019).

Figure 5. HRP breaks down the hydrogen peroxide produced by GOx into water and oxidizes ABTS (St. Denis, 2019).

Assay Sensitivity-Preliminary Test

- 10 µL and 20 µL of sweat compared to 10 µM glucose standard on the SpectraMax UV-Vis spectrophotometer to measure the change in absorbance for 20 minutes
- This was completed to determine if as little as 10 µL of sweat would generate a response when reacted with the enzymatic assay

Fluorescence Spectroscopy

- To further confirm the validity of the UV-Vis data, fluorescence spectroscopy was conducted using the Tecan i-control
- Sodium acetate buffer, GOx, and HRP were used in the same capacity, but instead of using ABTS, Ampliflu dissolved in dimethyl sulfide was used to correlate the amount of hydrogen peroxide produced into a measurable, fluorescent response
- 3 sweat and blood samples were used with the same time parameters (fasted, 1 hour after 16 g glucose, 2 hours after)
- Two systems were compared with a separate UV-Vis spectrophotometry test using the same samples provided

Blood Glucose and Sweat Collection Procedure

Voluntary participation with informed consent obtained from participants. All subjects required to be over the age of 18 and residents of the United States. Each asked to arrive in a fasted state for their 3-4 hour appointment for blood and sweat sample collection.

Sweat Samples taken via Pilocarpine Iontophoresis:
Sample 1: Fasted
Sample 2: 1 hr after consuming 16g glucose
Sample 3: 2 hr after consuming 16g glucose

Figure 6. Pilocarpine iontophoresis process (Brown, 2019).

Blood Samples taken:
Via finger prick, analyzed with glucometer at 15 min.

Pilocarpine Iontophoresis: Sweat Collection (Figure 6)
Two limb EKG electrodes secured to forearm

- Positive electrode: saturated with a 0.2% pilocarpine nitrate solution and placed on forearm closer to the wrist
- Negative electrode: saturated with a 0.07 N sodium bicarbonate solution and placed on forearm closer to the elbow
- Current raised slowly over 5-10 seconds to 2 milliamps and remains there

After 10 minutes of this sweat gland stimulation, electrodes were removed. New gauze was secured in place of positive electrode for 30 minutes of sweat collection

RESULTS

Assay Sensitivity-Preliminary Test

The sample of 10 µL of sweat did in fact generate a response in the system close to that of the 10 µM of glucose (Figure 7)

- Results indicate small samples of sweat are functional in system

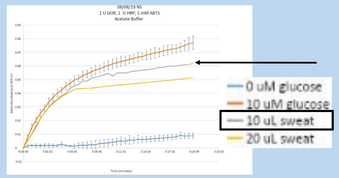

Figure 7. Change in absorbance at 405 nm over time (mins). Blank sample (DI water): blue line. Yellow line: 20 µL sweat sample. Grey line: 10 µL sweat sample. Orange line: 10 µM glucose standard (St. Denis, 2019).

Fluorescence Spectroscopy

Fluorescent reaction showed the same trend as the UV-Vis spectrophotometer reactions (Figure 9)

- Results indicate enzymatic assay is reliable in multiple detection systems

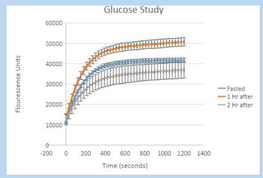

Figure 9. Fluorescence over time (seconds). Fasted sample: blue line. 1 hour after 16 g of glucose have been consumed: orange line. 2 hours after: grey line (Eldridge, 2019).

Blood Glucose and Sweat Collection 1

The first blood/sweat test generated a significant trend with blood glucose and sweat levels shown to be correlated (Figure 8)

- Sweat Sample 2 generated the highest absorbance as desired, because its corresponding blood glucose level (Table 1) was also the highest

Fasted	1 hour after 16g glucose	2 hours after 16g glucose
10 µL sweat per well	10 µL sweat per well	10 µL sweat per well
75 mg/dL blood glucose	107 mg/dL blood glucose	69 mg/dL blood glucose

Table 1. Sweat collected and the corresponding blood glucose levels (St. Denis, 2019).

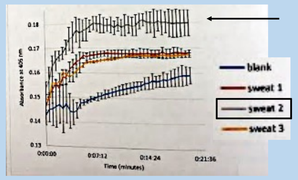

Figure 8. Absorbance at 405 nm over time (mins). DI water: blue line. Fasted sample: red line. 1 hour after consuming 16g of glucose: grey line. 2 hours after consuming 16g of glucose: yellow line. (St. Denis, 2019).

CONCLUSIONS

- After testing the sensitivity of the enzymatic assay with 10 µL and 20 µL of sweat
  - The results indicate this system is very sensitive and does not require a large amount of sweat in order to work
- Correlation was found between blood glucose levels and sweat:
  - Highest absorbance occurred at the highest blood glucose level (1 hour after consumption of 16 g glucose) and its corresponding sweat sample
  - Same output is expressed for other blood glucose levels and their corresponding sweat samples
- Fluorescence test generated the same trend as the UV-Vis spectrophotometer test
  - These results indicate greater sensitivity and reproducibility, which is very promising for future studies
- This novel, non-invasive technique for diabetics has shown to be a reliable means of detecting glucose levels

FUTURE WORK

- Testing the sensitivity of this system with even smaller amounts of sweat (as low as 1 µL), to make this novel technique even more efficient
- Focusing on fluorescence spectroscopy further with smaller amounts of sweat, as this has shown to be a promising method for this system
  - Generate fluorescence spectroscopy calibration curve
- Larger sample population to evaluate the functionality of this system with multiple people's sweat
- Identify possible devices this concept could be used in, such as a new form of a continuous glucose monitoring system

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