Amperometric Detection of Ultrasound-Induced Secretory Events in Potential Treatment of Type 2 Diabetes

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PURPOSE

Type 2 diabetes mellitus is a complex metabolic disease that has now reached epidemic proportions in the United States. Current pharmacological treatments are very complex and include many side effects which can result in further complications. The objective of this study is to explore a potential new treatment method that utilizes the non-invasive application of ultrasound energy to induce exocytosis of insulin from pancreatic β cells.

TYPE 2 DIABETES MELLITUS

- **Type 2:** Systemic insulin resistance, β-cell dysfunction and insufficient β-cell mass due to genetic and environmental factors.

Prevalence of Type 2 Diabetes

- In 2012, 27.85 million Americans were diagnosed with type 2 diabetes.
- 2010 to 2012 saw the emergence of 3 million cases of diabetes.
- Diabetes remains the 7th leading cause of deaths in the United States.

Cost of Type 2 Diabetes

- In patients diagnosed with type 2 diabetes at age 25-44, the lifetime cost of medical treatment is $124,700 and $130,800 for men and women respectively.

Fig. 1: Courtesy of the National Institute of Diabetes and Digestive and Kidney Diseases (NIH)

SPECIFIC AIMS

- Determine effectiveness of ultrasound stimulation of insulin release from pancreatic β-cells.
  - Detection of exocytotic secretion from beta cells.
  - Explore two different experimental parameters.
- Explore the pressure and thermal conditions cells are subjected to under experimental conditions.

ELECTRODE MANUFACTURING

1. A 7 µm carbon fiber thread was inserted into a 0.60 mm diameter capillary tube via vacuum suction.
2. Using a Sutton electrode puller, the threaded capillary tubes were pulled in half to create the taper around the carbon fiber.
3. The tapered end of the electrode was sealed with epoxy to prevent electron leakage and reduce noise during experimentation.
4. The open end of the capillary tube was filled with 3 M KCl, which acted as an electrical connection between the carbon fiber and wire.
5. Ag/AgCl wire attached to a connector pin was then inserted into the opn end. Epoxy and shrink tube were used to seal the back end.
6. As seen in Figure 3, the house made electrodes recorded an accurate reading of a known signal in a KCl solution.

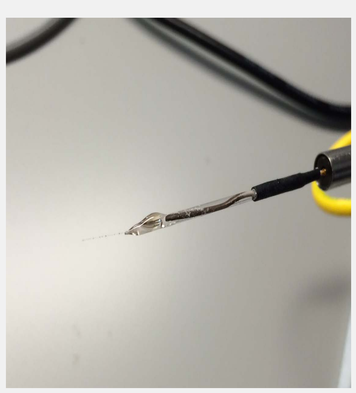

Fig. 2: Manufactured Electrode

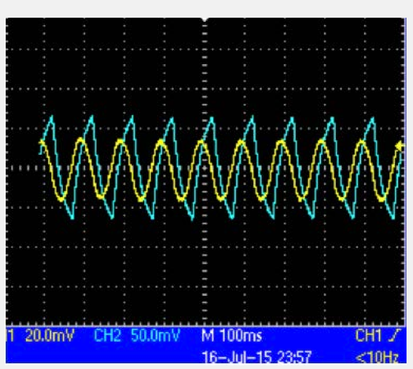

Fig. 3: Verification Plot– Blue (waveform generator), Yellow (electrode recording)

METHODS

Secretion Detection

- Exocytotic secretion from β-cells was detected using carbon fiber amperometry.
  - Commercially available electrodes were obtained from Kation Scientific.
- Ag/AgCl reference electrode was held at 650 V to produce sufficient potential difference such that a current was generated when dopamine was oxidized.

EXPERIMENTAL SETUP

- Pancreatic β-cells were loaded with dopamine and plated on polyethylene wells in a modified Krebs Bicarbonate Solution (KBS).
- Planar ultrasound transducer was used to stimulate pancreatic β-cells.
  - Center frequency: 800 kHz and 1 MHz
  - Intensity: 1 W/cm^2
- Transducer was positioned at the point between the transducer's near and far field distance (d_{FF} distance).
- Ultrasound was applied to the well at three minute intervals for up to 12 minutes either in 100 cycle bursts or short pulses of continuous ultrasound.

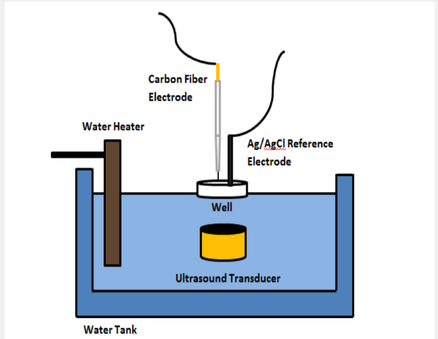

Fig. 4: Diagram of the experimental setup

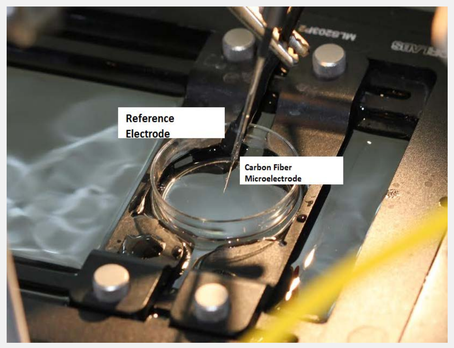

Fig. 5: Image of the carbon fiber electrode and reference electrode position in the well

SIMULATIONS OF ACOUSTIC FIELDS

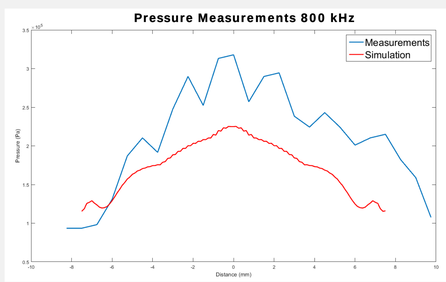

Fig. 6: Simulated vs. Measured Pressure Values for 800 kHz

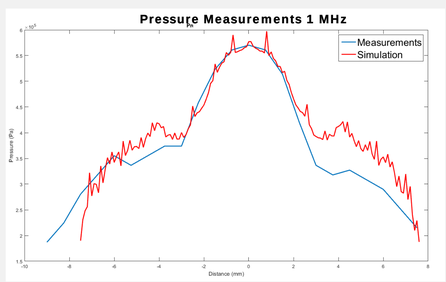

Fig. 7: Simulated vs. Measured Pressure Values for 1 MHz

Pressure measurements conducted with a lipstick hydrophone. These measurements were used to verify simulations of the acoustic fields experienced by the cells that were preformed in the modeling software, PZFLEX. The maximum pressures cells were exposed to was _____ and 5.95 x 10^5 Pa for 800 kHz and 1 MHz respectively.

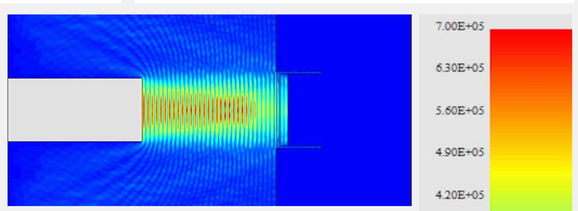

Fig. 8: 800 kHz PZFlex Pressure Simulation

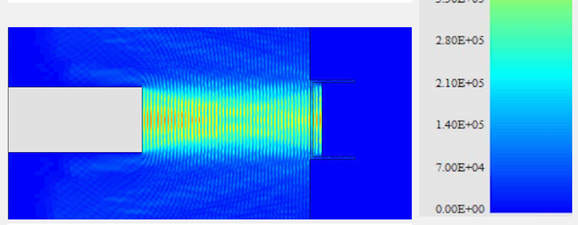

Fig. 9: 1 MHz PZFlex Pressure Simulation

RESULTS

Preliminary results indicate that application of ultrasound to pancreatic β cells leads to exocytosis. Continuous US pulses of varying durations produced sustained responses proportional to the duration of the pulse.

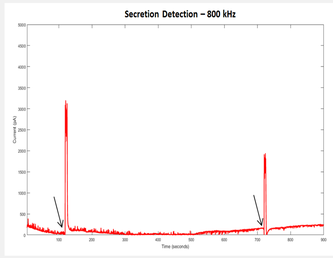

Fig. 10: Five s. 800 kHz continuous ultrasound pulses applied at 120 and 720 s.

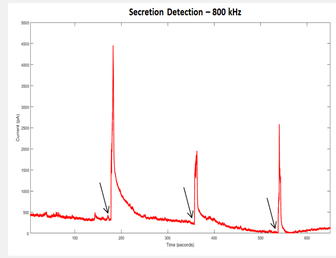

Fig. 11: Five s. 800 kHz continuous pulses applied at 180, 360 and 540 s.

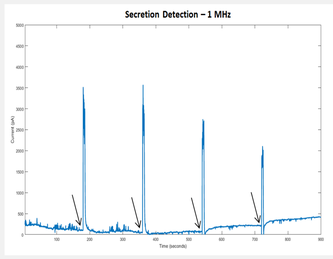

Fig. 12: Five s. 1 MHz continuous pulses applied at 180, 360, 540 and 720 s.

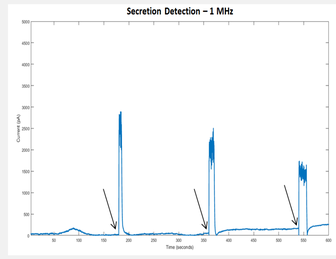

Fig. 13: Five, ten and fifteen s. 1 MHz continuous pulses applied at 180, 360 and 540 s.

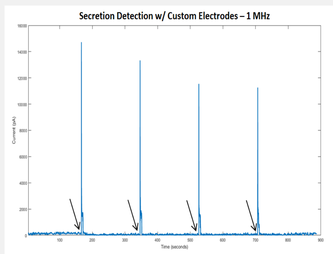

Fig. 14: Five s. 1 MHz continuous pulses applied at 180, 360, 540, and 720 s. Measured with custom electrodes.

- Experiments conducted utilizing custom electrodes produced similar results to those performed with commercially available electrodes.
- Experiments conducted without any dopamine loaded into the cells showed no results.
- Positive control experiments are still ongoing.

CONCLUSION AND FUTURE STUDIES

Low intensity ultrasound remains a potential treatment that directly targets one of the underlying causes of insulin deficiency in Type 2 diabetes. If proven successful, the non invasive nature of ultrasound treatment could present an alternative to current medications in the treatment of Type 2 diabetes mellitus. Future studies will look at how different parameters (intensity, pulse length) would affect the amount of insulin released. Consequently, *in vivo* studies will be done to further explore how the changes of certain parameters affect the pancreas wholly (in terms of potentially stimulating other endocrine cells).

POTENTIAL PRODUCT

Fig. 15: Potential Product

Our potential product consists of a planar US transducer that can be strapped to the back of a patient. The parameters of US delivery are controlled by a wireless remote which also functions as a glucose monitor to allow the patient to optimize insulin release.

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Acknowledgment: Funded by NIH Award-1R03EB019065-01