Ultrasound for correction of secretory deficiencies as a potential novel treatment for type 2 diabetes

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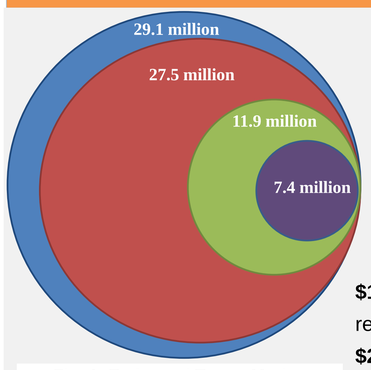
PURPOSE

Type 2 diabetes mellitus is a complex metabolic disease that has reached epidemic proportions in the United States and around the world. Controlling type 2 diabetes is often difficult. Many patients are poorly compliant with lifestyle change recommendations, and pharmacological management routinely requires complex therapy with multiple medications, and loses its effectiveness over time. Thus, new modes of therapy are needed that will target directly the underlying causes of abnormal glucose metabolism. The objective of this study is to explore a novel, non-pharmacological approach that utilizes the application of ultrasound energy to augment insulin release from pancreatic β-cells.

THE COST OF DIABETES



$176 billion spent on direct medical costs related to diabetes in the US in 2012

$2.3 billion spent on insulin secreting drugs

Fig. 1: Estimated Target Market

POTENTIAL PRODUCT

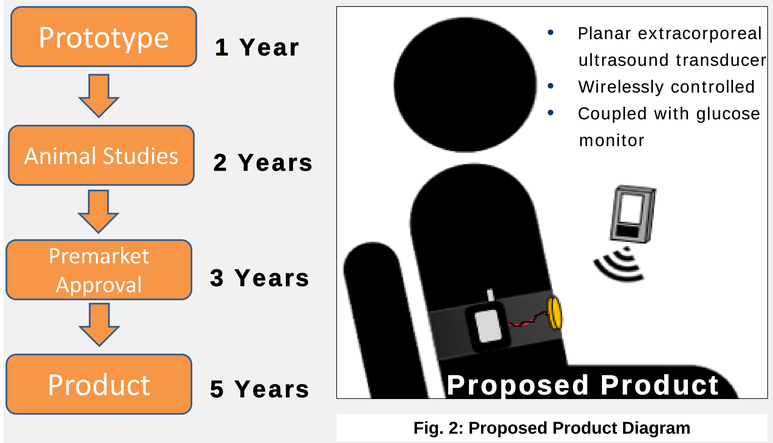


Fig. 2: Proposed Product Diagram

SPECIFIC AIMS

- Determine effectiveness of ultrasound stimulation of insulin release from pancreatic β-cells.
  - ➢ In this aim, we test the effectiveness of low-intensity ultrasound at different parameters in stimulation of insulin release from pancreatic β-cells.
- Determine effects of ultrasound stimulation on viability of the pancreatic β-cells.
  - ➢ In this aim, we test the extent to which ultrasound stimulation affects viability of human pancreatic β-cells.
- Explore the mechanisms involved in ultrasound-enhanced insulin release from pancreatic β-cells.
  - ➢ In this aim, we use a simulation software called PZFlex to model our experimental setup and create pressure maps of the applied ultrasound exposures. Temperatures inside the chamber are monitored experimentally with a thermocouple.

METHODS AND MATERIALS

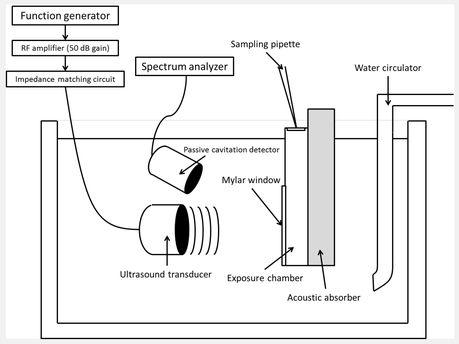


Fig. 3: Experimental Setup 1

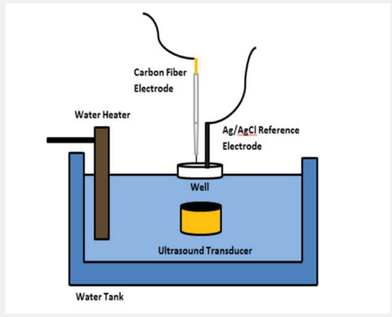


Fig. 4: Experimental Setup 2

- A planar ultrasound transducer was used to sonicate the cells using a wide range of ultrasound parameters.
  - Frequencies: 400 k Hz, 600 kHz, 800 kHz, 1 MHz
  - Intensites: 0.5, 1 W/cm^2
  - Duty cycle: 100% (continuous) for a duration of 5 minutes
- β-cells were placed and suspended in an exposure chamber made of polylactic acid (PLA) with acoustic transparent windows made of Mylar (**Fig. 3**).
- Cell samples (100 µL) were collected before ultrasound treatment (t = 0 min), 5 min after the start of the treatment and 30 min after the end of treatment.
- In a separate set of experiments, we used amperometry detection to test controllability of ultrasound-stimulated secretory release from β-cells (**Fig. 4**)

PRESSURE MAPS & THERMAL MEASUREMENTS

- The experimental setup was modeled with PZFlex and pressure maps were generated for all ultrasound exposures to determine the range of pressures to which the cells are exposed to during treatment **(Fig. 5).**
- Material properties, parameters and dimensions were compiled from measurements and manufacturers' data.
- Temperature variations inside the exposure chamber were monitored throughout the ultrasound treatment by inserting a thermocouple in the chamber.
- In all cases, temperature inside the chamber increased by a maximum 2°C on average (**Fig. 6**).

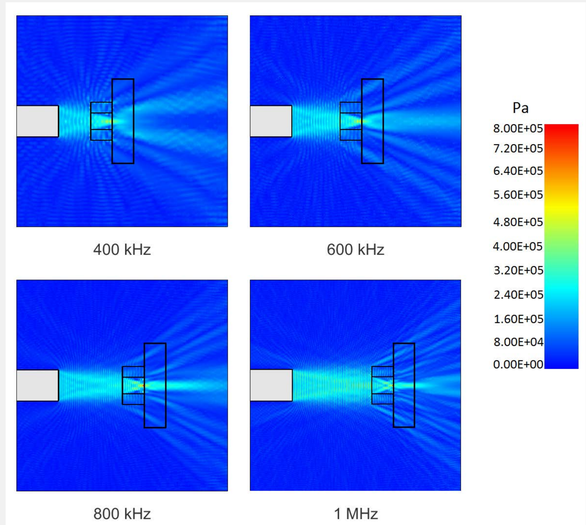


Fig. 5: Pressure maps of all ultrasound exposures generated with PZFlex modeling software

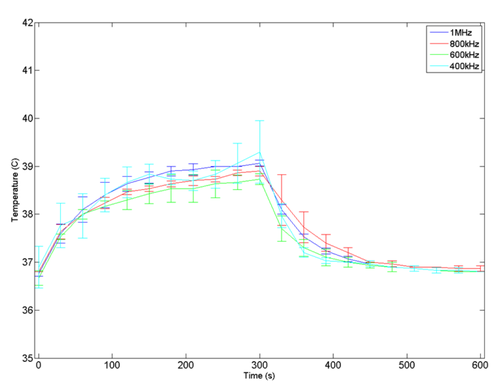


Fig. 6: Temperature variations inside the exposure chamber for all ultrasound exposures

RESULTS

- Our data indicated that application of therapeutic ultrasound can lead to safe increase of insulin secretion from β-cells at a frequency of 800 kHz and intensity of 1 W/cm^2.
- Amperometry results suggest that ultrasound-stimulated secretory events can be tightly controlled in a Ca^{2+}-dependent manner.

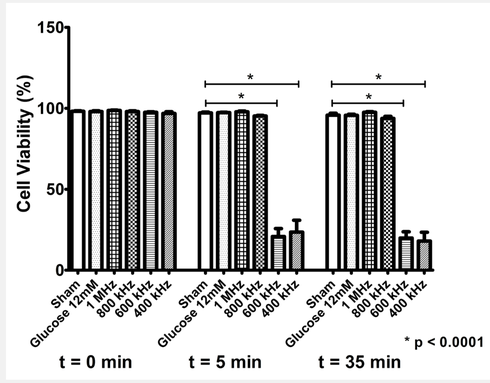


Fig. 7: Results (n=6) of cell viability after ultrasound treatment as measured by trypan blue dye exclusion test.

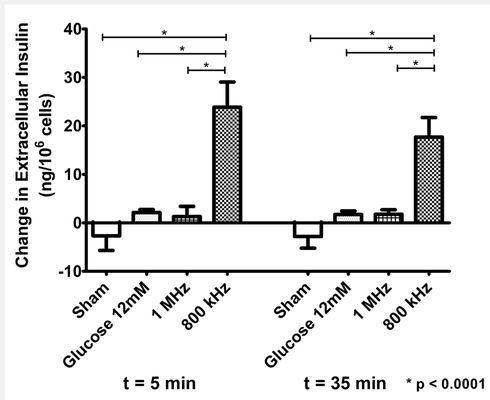


Fig. 8. Results (n=6) of insulin released into the extracellular space by β-cells exposed to ultrasound as measured by Insulin ELISA. Measured insulin values at t = 5 min and t = 35 min were normalized to initial values measured at t = 0 min.

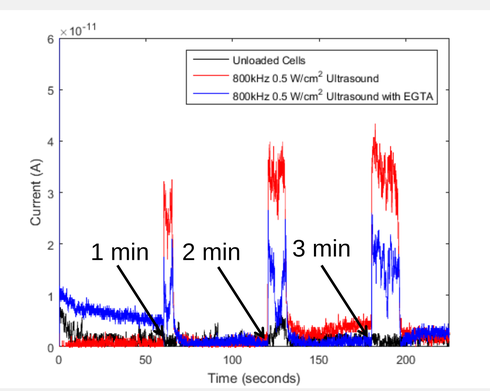


Fig. 9. Five, ten and fifteen sec long 800 kHz continuous pulses applied at 60, 120 and 180 sec. Amperometric detection of neurotransmitter release mimics the secretion dynamics of insulin in beta cells in a Ca^{2+}-dependent manner.

CONCLUSIONS AND FUTURE WORK

If proven successful our method may find a clinical application due to the non-invasive nature of therapeutic ultrasound treatment of human pancreas (through an appropriate acoustic window). Our future studies will focus on finding an optimal set ultrasound parameters for applications to the pancreas in an *in vivo* animal model, to determine whether it would be possible to stimulate β-cells without stimulating other endocrine and exocrine cells of the pancreas.

Acknowledgment: Funded by NIH Award-1R03EB019065-01

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