ULTRASOUND STIMULATION OF INSULIN RELEASE FROM PANCREATIC BETA CELLS

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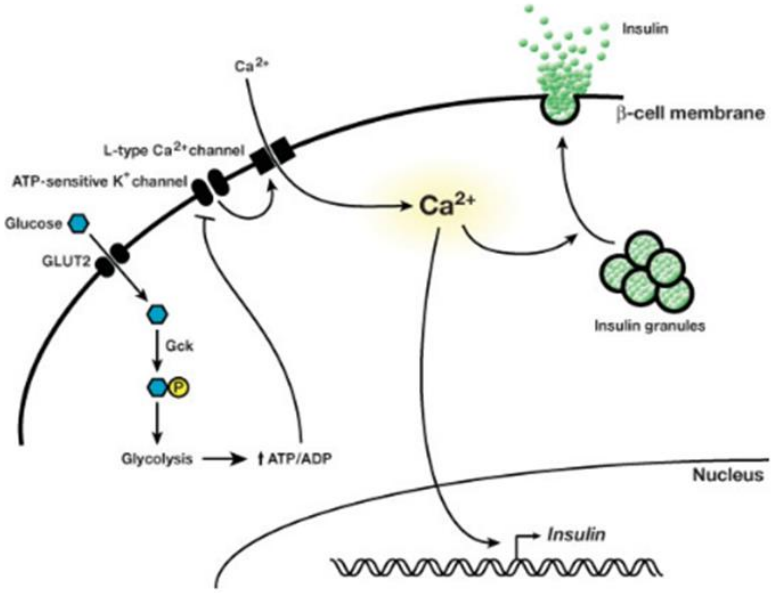
Motivation

- 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 as 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.
- The objective of this study is to explore a novel, non-pharmacological approach that utilizes the application of ultrasound (US) energy to augment insulin release from pancreatic β-cells.

Background

- Pancreatic β-cells in the islet of Langerhans secrete the hormone insulin, which is required for systemic control of glucose.
- Insulin is released from the β-cells in a calcium-dependent manner (Rorsman et al. 2012). It has been suggested that Ca^{2+} signaling is both necessary and sufficient for glucose-stimulated insulin release.

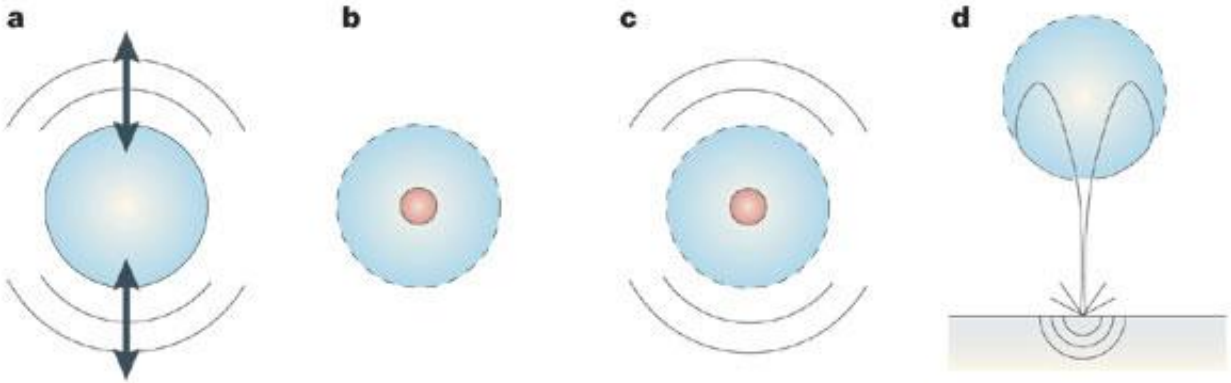


Schematic illustrating Ca^{2+} regulation of insulin secretion, transcription and release (Rorsman et al. 2012).

Background (2)

Ultrasound induced-bioeffects have been widely shown to produce intracellular calcium transients in various cell types. (Wu et al. 2008, Zarnitsyn et al. 2008, Hassan et al. 2010).

- Cells exposed to acoustic cavitation and other mechanical stresses can be transiently permeabilized by the formation of pores that can be "self-sealed" by the cell. (Wu et al. 2008, Zarnitsyn et al. 2008).

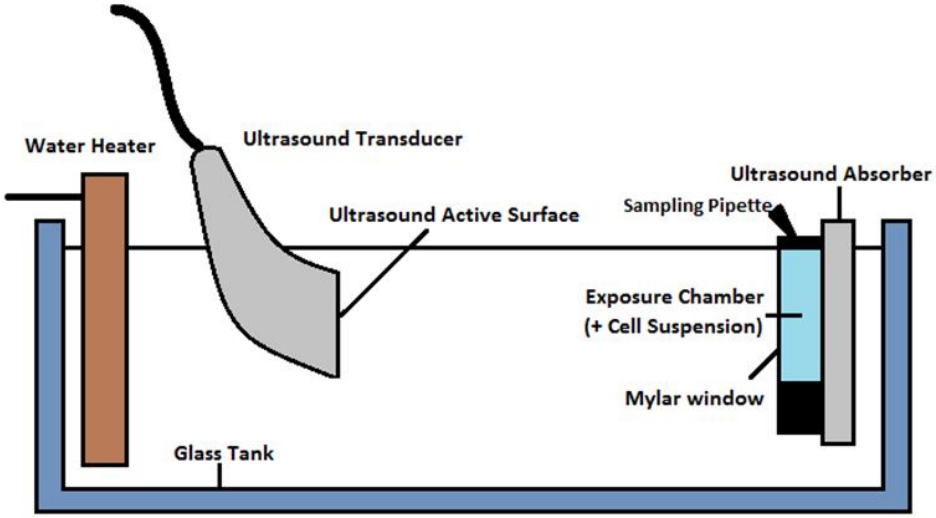


Ultrasound Cavitation. Nature (2012)

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 human islets of Langerhans.
  - In this aim, we test the extent to which ultrasound stimulation affects viability of human pancreatic β-cells.

Methods and Materials



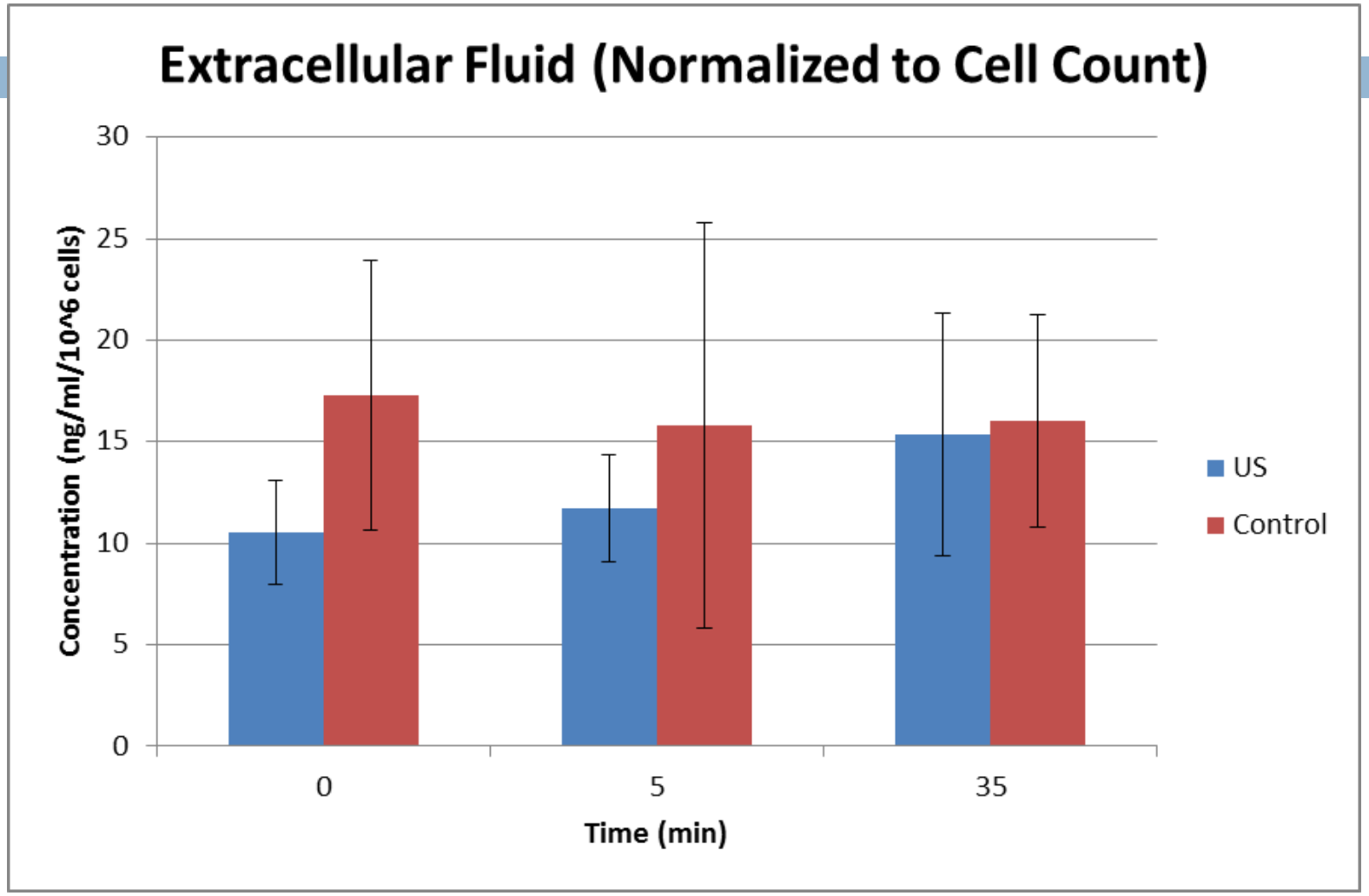
Experimental setup for ultrasound stimulation of pancreatic β-cells.

- A planar ultrasound transducer was used to sonicate the cells using a wide range of ultrasound parameters.
  - Center frequency: 1 MHz
  - Intensity: 1 W/cm^2
  - Duration: 5 min
- INS-1 β-Cells suspended in Krebbs Ringer Buffer and placed in an exposure chamber made of polylactic acid (PLA) with acoustic transparent windows made of Mylar.
- The exposure chamber containing the cell suspension was filled with fluid and was placed at the acoustic focus of the transducer (DFF distance).
- Cell samples (100µL) were collected before US treatment (t = 0 min), immediately after treatment (t = 5 min) and 30 minutes after treatment for analysis.

Cell Sample Analysis

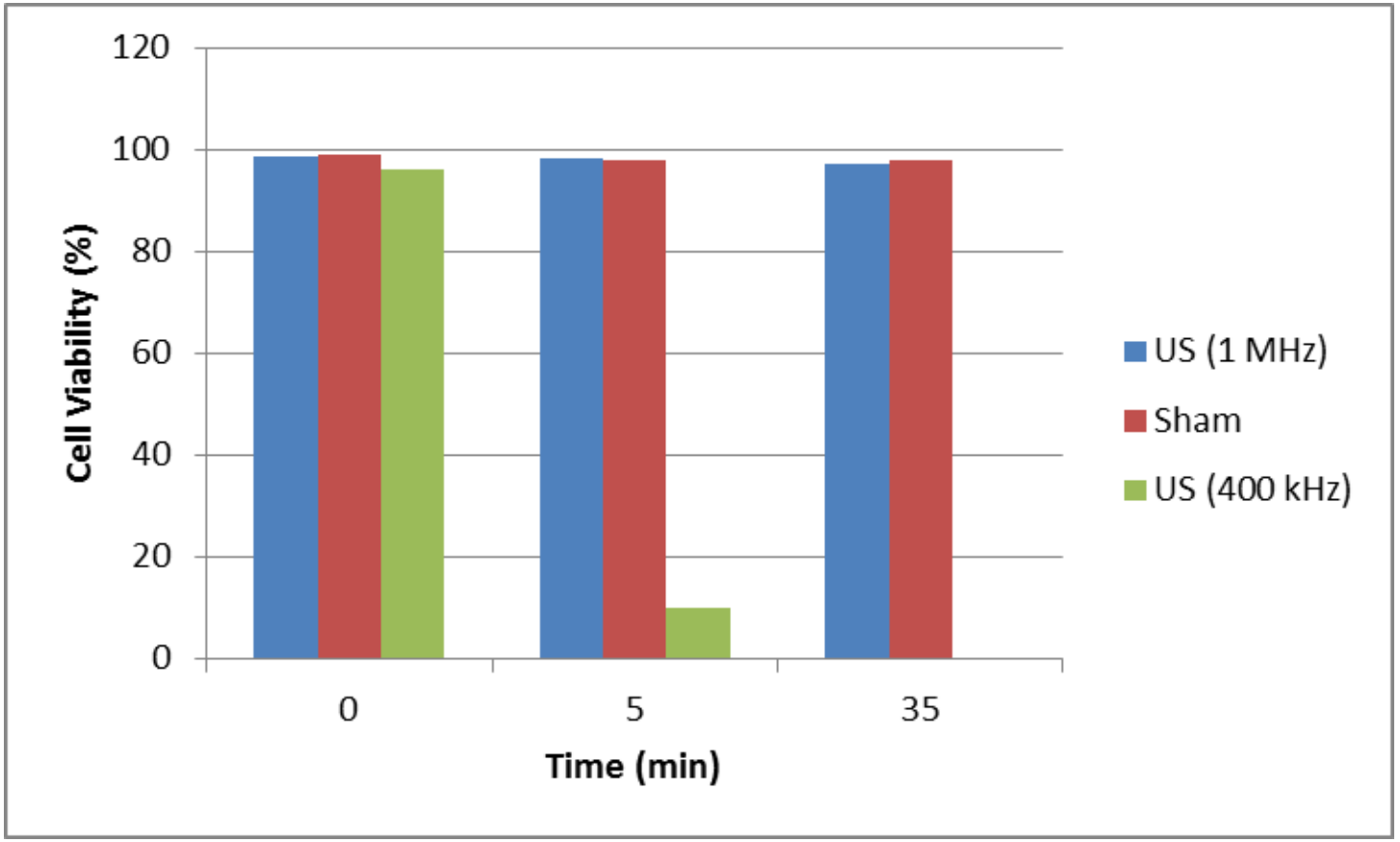
- Quantification of Insulin Release:
  - Using ELISA insulin release assay, we determined effects of ultrasound-induced insulin release from suspended β-cells.
- Cell Viability Studies:
  - Cell viability was quantified using trypan blue and an automatic cell counter. Results were expressed as the ratio of counted live cells to the total cell count (%).

Preliminary Insulin Release Results



Preliminary results (n=3) of insulin release from β-cells after ultrasound (US) exposure for 5 min and 35 min (mean ±SD)

Preliminary Cell Viability Results



Low-intensity ultrasound application at 1MHz was not detrimental to cultured INS-1 pancreatic β-cells. (n=3)

Conclusions and Future Work

- Our preliminary results show no significant effects of ultrasound in increased insulin secretion from pancreatic beta cells. Cell viability was shown not to be affected by ultrasound exposure at 1 MHz, 1 W/cm^2 for a duration of 5 min.
- Future work will include conducting experiments at different ultrasound parameters (i.e. different frequencies, intensities) and application of this treatment on pancreatic islets.

References

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Acknowledgment

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