Purpose:

The objective of this study was to analyze the gene expression of Klotho in C2C12 cells using RT-qPCR

Electrical Signals:

The C2C12 cells were stimulated for 60 minutes, 300 us continuous cycle using a Mettler stimulator at various frequencies and currents ranging from 0.5, 2, 5 mA.

Methods:

Bioelectric stimulation was applied to cells *in vitro* using a commercially available Mettler stimulator via a 6-well stimulating plate interface (IONOPTIX, Westwood, MA, USA). To induce uniform electric fields in all stimulation chambers, 1.3 mL of DMEM solution was added to each well before BES signal application.

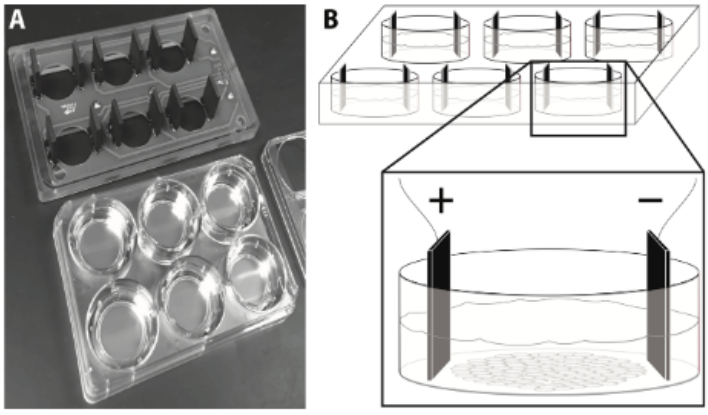
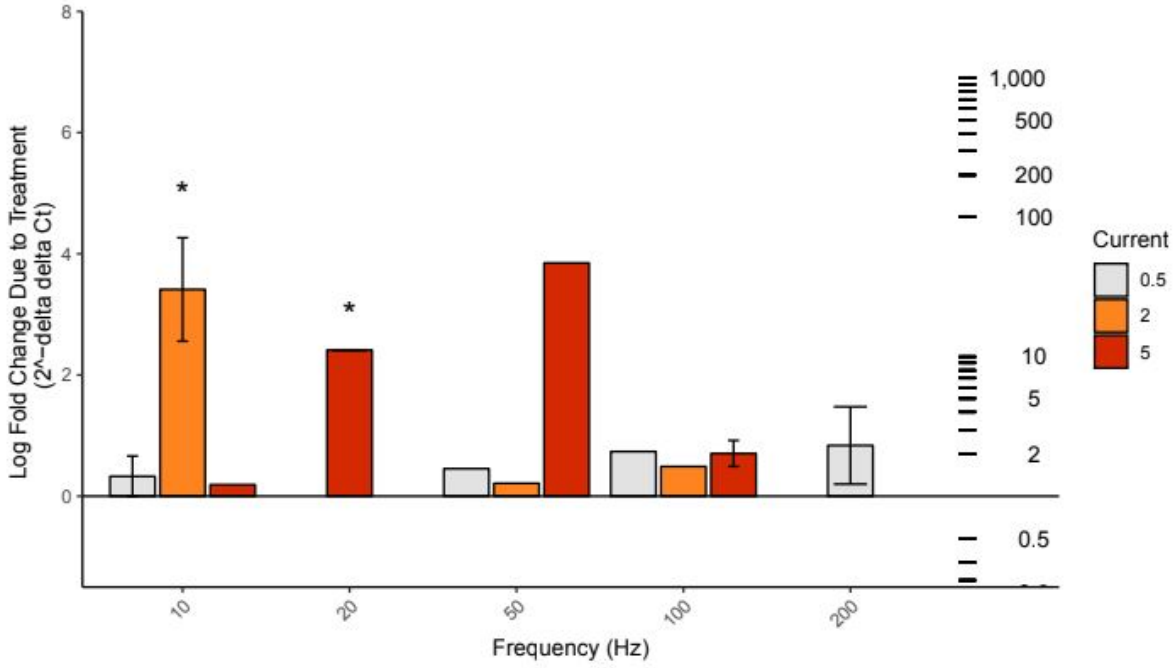


***Figure 1**. Bioelectric Stimulation System. Cells were plated in each dish and cultured to 80% - 100% confluency. Once confluent, cells were stimulated using an electrode array (shown at the top of panel A), which was inverted and introduced into the 6-well dish where cells were grown. Each well received uniform stimulation via a pair of carbon electrodes positioned at opposite sides (panel B).*

Gene expression was determined by extracting mRNA according to the manufacturer's instructions. RNA quality was determined using a spectrophotometer and was reverse transcribed using a cDNA conversion kit. The cDNA and TaqMan Master mix was used.

Results:



Right tick marks indicate fold change.

.* Indicates significant change due to treatment.

Figure 1. Klotho gene expression in C2C12 cells stimulated for 60 minutes.

Table 1. Gene expression of C2C12 cells normalized to GAPDH using RT-qPCR.

KLOTHO normalized by GAPDH - Fold change due to treatment

	Frequency	Current	N	Fold	sd	se	ci	ci.Lower	ci.Upper
1	0	0.0	2	1.08	0.59	0.42	5.29	-4.21	6.37
2	10	0.5	3	1.53	0.74	0.43	1.85	-0.32	3.38
3	10	2.0	3	63.26	86.22	49.78	214.19	-150.93	277.45
4	10	5.0	1	1.21	NA	NA	NaN	NaN	NaN
5	20	5.0	2	11.16	0.03	0.02	0.30	10.87	11.46
6	50	0.5	1	1.57	NA	NA	NaN	NaN	NaN
7	50	2.0	1	1.24	NA	NA	NaN	NaN	NaN
8	50	5.0	1	46.93	NA	NA	NaN	NaN	NaN
9	100	0.5	1	2.09	NA	NA	NaN	NaN	NaN
10	100	2.0	1	1.63	NA	NA	NaN	NaN	NaN
11	100	5.0	2	2.07	0.62	0.44	5.57	-3.50	7.64
12	200	0.5	2	2.80	2.23	1.58	20.03	-17.23	22.82

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Conclusions:

After adjustments, one-sample T-Test showed that KLOTHO increased in the following conditions: 10 Hz at 2 mA, 20 Hz at 0.5 and 5 mA, and 50 Hz at 5 mA (which increased KLOTHO 6088 fold). The ANOVA

revealed Current influenced the magnitude of increase of KLOTHO, likely meaning 0.5 mA had lower increases in KLOTHO than 2 and 5 mA.