Analysis of Spontaneous Calcium Signals to Infer Functional Connectivity Within A Novel "Living Electrode" Neural Construct

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Micro-Tissue Engineered Neural Networks (uTENNs) are novel, living, neural constructs, designed to be implanted into the central nervous system (CNS) to treat neurological disease and injury. Each uTENN is a implantable self-assembly of seeded neurons in three dimensional, micro-column hydrogel scaffolds. uTENNs can be a new strategy to facilitate nervous system repair. With an externalized pole positioned under optoelectronic arrays at the cortical surface, the uTENN functions as a "living electrode" with its neurons forming synapses in the surrounding host brain to modulate activity.

The goal of this project was to characterize uTENNs *in vitro* through a functional analysis of spontaneous calcium signals, a marker of neural firing rate. We acquired the spontaneous calcium signals of neurons in individual uTENNs *in vitro* using the genetically encoded calcium indicator GCaMP6f ($\lambda_{excitation}$: ~450nm; $\lambda_{emission}$: ~510nm) via a Nikon Eclipse Ti microscope on the NIS Elements software platform. The uTENN used in this study was a 2.0 mm agarose tube with an outer diameter of 345 μm and an inner diameter of 180 μm, filled with an extracellular matrix consisting of collagen and laminin; this construct was seeded at both ends with clusters of embryonic day 18 rat cortical neurons that had been transfected to express GCaMP. After nine days *in vitro*, this microTENN was imaged for 120 seconds at 20 frames per second (2400 frames acquired).

The acquired calcium signals were then analyzed using a suite of Matlab toolboxes: Fluorescence Single Neuron and Network Analysis Package (FluoroSNNAP), EEGLAB , and our own customized code. We took advantage of the interactive graphic user interface (GUI) segmentation, and event-detection modules of FluoroSNNAP to identify regions of interest (ROIs), which are neurons with calcium signals. A matrix that included calcium signals of all selected ROIs was generated. We used EEGLAB to generate Granger Causality Connectivity, to indicate information flow and hence functional connectivity among neurons, and our own code to produce Pearson Cross Correlation, Phase Synchronization.

In an analysis of 38 neurons made in one recording of this particular uTENN, we found that the neurons were correlated and synchronized, implying functional connectivity. The majority of the elements in the Pearson Cross Correlation matrix had a value above 0.8, which showed that the majority of neurons in uTENN were highly correlated. Similarly, the majority of the elements in Phase Synchronization matrix had a value above 0.8, which indicated high synchronicity. Analysis of Granger Causality Connectivity matrix showed a moderate volume of information flow and therefore implied that the neurons in uTENN are functionally connected.

The correlated, synchronized, and functionally connected neuronal activities in this uTENN support further investigation of using uTENNs in treating human neurodegenerative diseases and CNS injuries. The methods and results of our functional analysis will be shown in greater details at the symposium.

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Abstract

Micro-Tissue Engineered Neural Networks (uTENNs) are novel, living, neural constructs, designed to be implanted into the central nervous system (CNS) to treat neurological disease and injury (1, 2). Each uTENN is a implantable self-assembly of seeded neurons in three dimensional, micro-column hydrogel scaffolds. With an externalized pole positioned under optoelectronic arrays at the cortical surface, the uTENN functions as a **"living electrode"** with its neurons forming synapses in the surrounding host brain to modulate activity. **The goal of this project was to characterize uTENNs *in vitro* through a functional analysis of spontaneous calcium signals, a marker of neural firing rate (3).** We acquired the spontaneous calcium signals of neurons in individual uTENNs *in vitro* using a genetically encoded calcium indicator (gCaMP). The acquired calcium signals were then analyzed using a suite of Matlab toolboxes: Fluorescence Single Neuron and Network Analysis Package (FluoroSNNAP), EEGLAB , and our own customized code. We took advantage of the interactive graphic user interface (GUI) segmentation, and event-detection modules of FluoroSNNAP to identify regions of interest (ROIs), which are neurons with calcium signals. A matrix that included calcium signals of all selected ROIs was generated. We used EEGLAB to generate **Granger Causality Connectivity**, to indicate information flow and hence functional connectivity among neurons, and our own code to produce **Pearson Cross Correlation**, **Phase Synchronization**.

Introduction and Relevance

The human brain has a limited ability to repair itself in response to large deficits imposed by stroke, traumatic brain injury, or chronic neurodegenerative diseases. uTENNs, as shown in **Figure 1** and **2**, has been shown to have the potential to be a new strategy to facilitate nervous system repair (4, 5). Our study is significant in uTENNs development. The neurons in uTENNs need to demonstrate **1) correlation, 2) synchronicity, and 3) functionally connectivity** before we can proceed to more *in vivo* studies to further investigate uTENNs' efficacy in repairing human CNS.

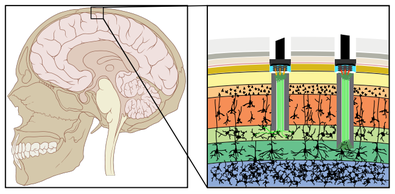


Figure 1: Schematic diagram of uTENN in human application.

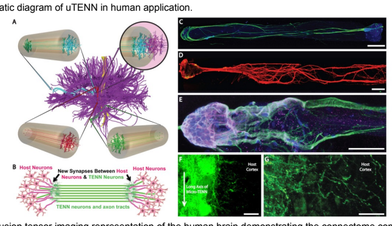
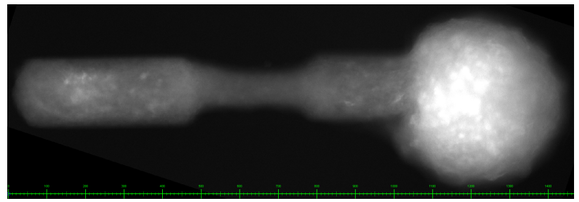


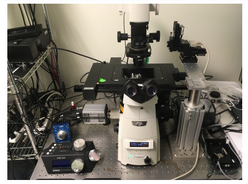
Figure 2: (A) Diffusion tensor imaging representation of the human brain demonstrating the connectome comprised of long distance axonal tracts connecting functionally distinct regions of the brain. Unidirectional (red, green) micro-TENNs and bi-directional (blue) micro-TENNs can bridge various regions of the brain (blue: corticothalamic pathway, red: nigostriatal pathway, green: entorhinal cortex to hippocampus pathway) and synapse with host axons (purple; top right). (B) Conceptual representation of a micro-TENN forming local synapses with host neurons to form a new functional relay to replace missing or damaged axonal tracts. (C) Confocal reconstruction of a bi-directional micro-TENN, consisting of two populations of neurons spanned by long axonal tracts within a hydrogel micro-column stained via immunocytochemistry to denote axons (b-tubulin III; green), and cell nuclei (Hoechst; blue). (D) Confocal reconstruction of a unidirectional micro-TENN, consisting of a single neuron population (MAP2; green) extending axons (Tau; red) longitudinally (5). (E) Confocal reconstruction of a unidirectional micro-TENN, stained via immunocytochemistry to denote neuronal somata/dendrites (MAP2; purple), neuronal somata/axons (Tau; green), and cell nuclei (Hoechst; blue). (F) Confocal reconstruction of a transplanted GFP+ micro-TENN showing lateral outgrowth in vivo. (G) Confocal reconstruction showing GFP+ processes extending from a transplanted micro-TENN into the cortex of a rat. Scale bars: 300 μm in C, 250 μm in D, 100 μm in E, 20 μm in F and G.

Methods (uTENN construction)

- 2.0 mm agarose tube
- Outer diameter of 345 μm
- Inner diameter of 180 μm
- Extracellular matrix consisting of collagen and laminin
- Embryonic day 18 rat cortical neurons seeded at both ends (GCaMP expression)
- 9 days *in vitro* before imaging

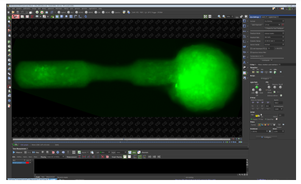


Methods (Signal Processing)

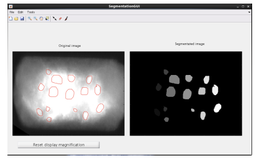


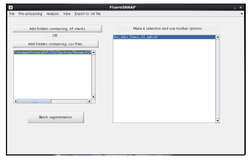
GCaMP Signal

$\lambda_{excitation}$:~450nm
$\lambda_{emission}$: ~510nm



uTENN 120-second image stack (.tif) 20 FPS

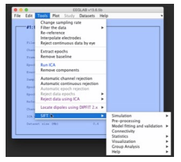




Fluorescence Single Neuron and Network Analysis Package (6)
ROIs identification

Customized MATLAB toolbox

EEGLAB (7)



Pearson Cross Correlation

$$r = \frac{\sum_i (x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_i (x_i - \overline{x})^2}\sqrt{\sum_i (y_i - \overline{y})^2}}$$

Phase Synchronization (8)

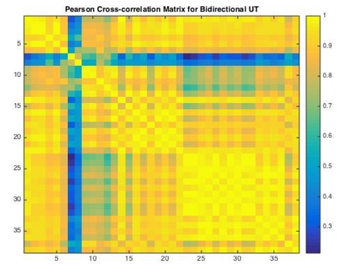
$$\mathrm{R}_{ij} = \left| \frac{1}{n} \sum_{l=1}^{n} \exp\left(\mathrm{i}\,(\phi_{jl} - \phi_{il})\right) \right|$$

Granger Causality Connectivity (9)

$$\mathrm{U_t} = \sum_{k=1}^{p} \mathrm{A_k U_{t-k}} + \varepsilon_t$$

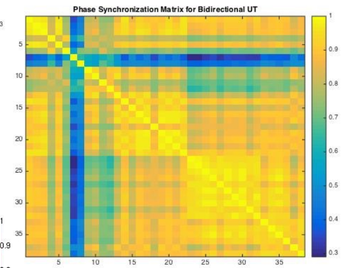
Results

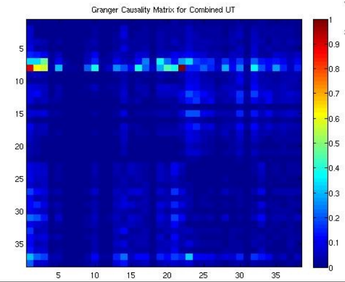
38 ROIs identified



>0.8 highly Correlated

>0.8 highly Synchronized





Moderate information flow
Functionally connected

Key Points

- Neurons in uTENNs exhibit correlated, synchronized activity
- Granger causality suggests information flow within the uTENNs
- Further study of uTENNs may inform optimal construct design and implantation strategies to better treat human CNS disease and injury

References, Disclosure, Acknowledgement

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