A modular platform to generate functional sympathetic neuron-innervated heart assembloids

Hsueh-Fu Wu[1, 2], Kenyi Saito-Diaz[1, 2], Xin Sun[3], Ming Song[4], Tripti Saini[1, 2], Courtney Grant[5], Christina James[1, 2], Kimata Thomas[1], Yohannes Abate[6], Elizabeth W. Howerth[7], Peter A. Kner[4], Bingqian Xu[3], Nadja Zeltner[1, 2, 8]

[1]Center for Molecular Medicine, UGA. [2]Department of Biochemistry and Molecular Biology, UGA [3]College of Engineering, UGA. [4]School of Electrical and Computer Engineering, UGA. [5]Department of Environmental Sciences, UGA. [6]Department of Physics and Astronomy, UGA. [7]Department of Pathology, UGA. [8]Department of Cellular Biology, UGA.

Department of Biochemistry and Molecular Biology
Franklin College of Arts and Sciences
UNIVERSITY OF GEORGIA

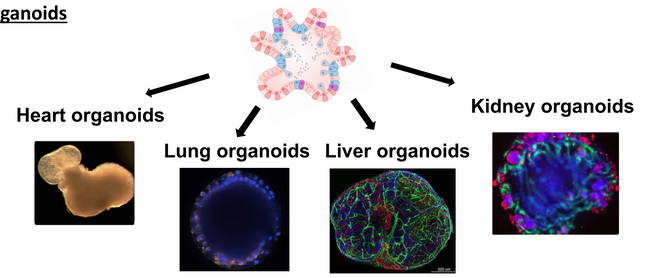
THE CENTER FOR Molecular Medicine

Abstract

The peripheral sympathetic nervous system (SNS) innervates and regulates the maturation and functionality of almost all organs in the body. One of the most vital organs that the SNS regulates is the heart. Sympathetic neuron (symN) signaling facilitates cardiac development, maturation, and increases heartbeat. SNS dysregulation has been linked to cardiac dysfunction, such as arrythmia and myocardial infarction. Human organoids derived from human pluripotent stem cells (hPSCs) are valuable tools in studying organ development and functionality in healthy and diseased states. However, despite a wide availability of cardiac organoid protocols, none of these organoids are symN-innervated, thus lacking the neurocardiac interaction. We have previously reported a well-established symN protocol using hPSCs, that has been applied to modeling multiple SNS diseases. Here, we developed an efficient strategy to make sympathetic neuron (symN)-innervated cardiac assembloids without the need for complex bioengineering approaches. Our human sympathetic cardiac assembloids (hSCAs) are self-organized, and showed cardiomyocyte maturation, cardiac cavity formation, atrial to ventricular patterning, and spontaneous beating. In hSCAs, we also observed symN innervation with neurotransmitter release, and regulation of the cardiomyocyte beating rate, which could be manipulated pharmacologically or optogenetically. Using this platform, we modeled symN-mediated early heart development and myocardial infarction. This easy-to-access and highly versatile modular platform will facilitate the study of neuron-organ interaction *in vitro* and may be applicable to make more assembloid models of variable organs (such as kidney and lung) with diverse peripheral neurons (such as parasympathetic and sensory neurons).

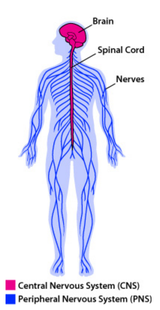
Introduction

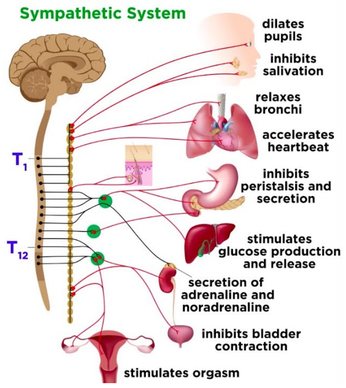
3D organoids



➡ Most organoids are not innervated

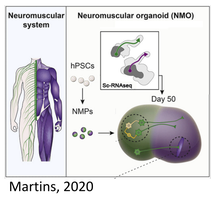
Sympathetic nervous system: biological function





Current methods to make innervated organoids

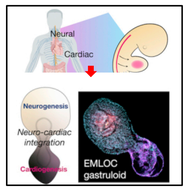
- **Multicompartment organoids**



Martins, 2020

Weakness
- Need common progenitors (e.g., NMP)

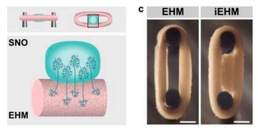
- **Early embryoids**



Olmsted, 2022

Weakness
- Time consuming
- Cell specification

- **Complex bioengineering**

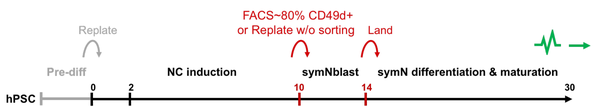


Schneider, 2023

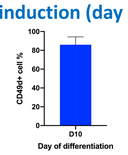
Weakness
- Specialized equipment
- Lack of 3D structures

Not flexible

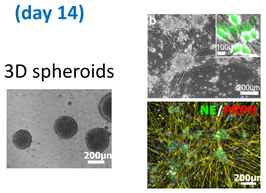
Human pluripotent stem cell-derived symNs and cardiac progenitors



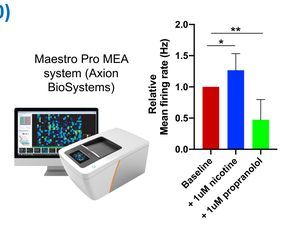
- **Efficient NC induction (day 10)**

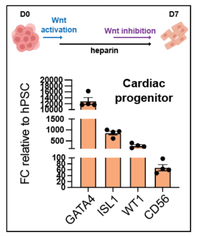


- **SymNblast induction (day 14)**
- **High-yield symN generation (day 30)**



- **hPSC-symNs are functional**

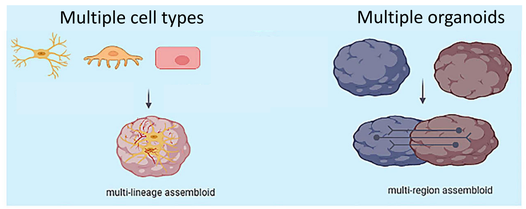




Specific aims

- **To develop ANS-innervated organ models**
- **A simple, easy to reproduce, efficient, modular platform**

  => Assembloid technology: self-organizing admixtures of ≥ 2 cell types/organoids (Kanton, 2022)



Makrygianni, 2021

Results

Figure 1. Building the organoid using the assembloid technology

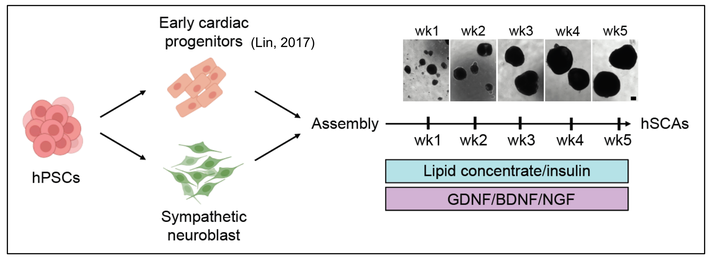
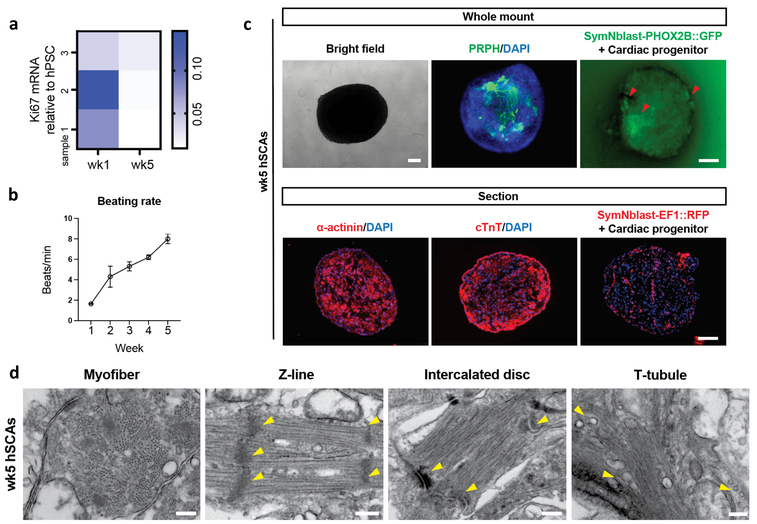
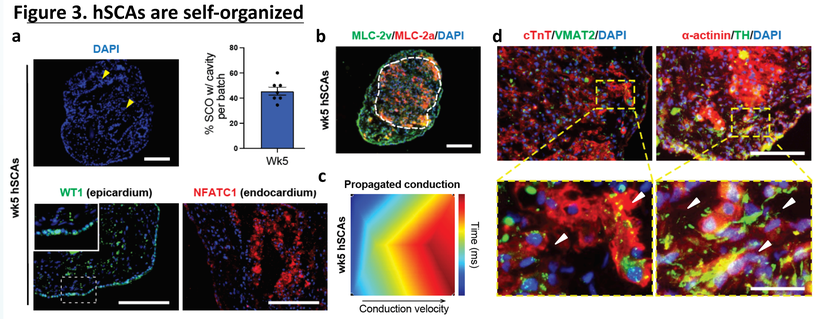


Figure 2. Characterization of hSCAs



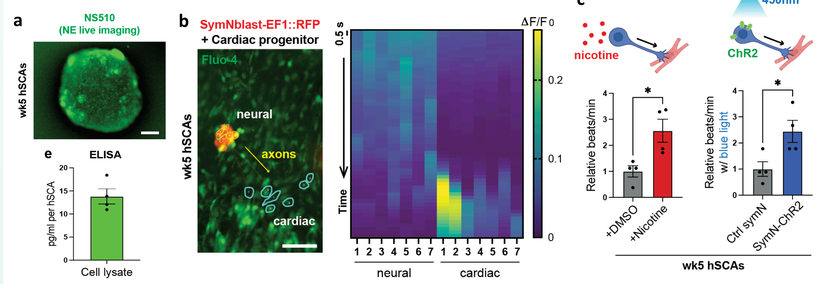
(a) Ki67 mRNA expression. (b) hSCAs beating over time. (c) IF staining. (d) Mature features by TEM

Figure 3. hSCAs are self-organized



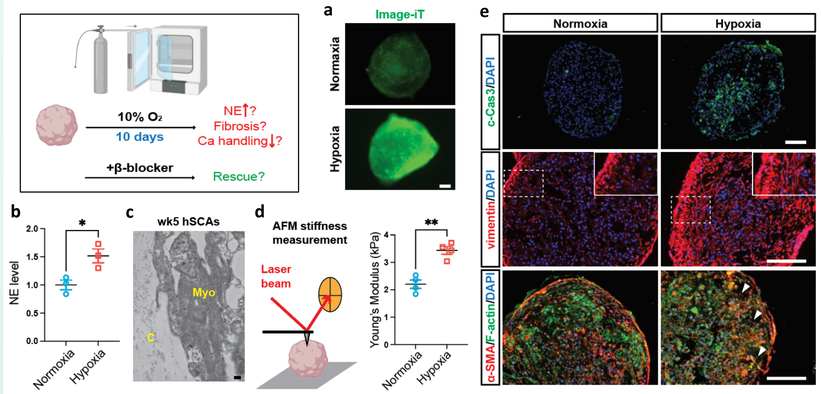
(a) Cavity structures in hSCAs. (b) Atrial-ventricular patterning in hSCAs. (c) MEA field potential measurement. (d) SymN axon innervation in hSCAs.

Figure 4. SymN regulation in hSACs



(a) NE release in hSCAs. (b) SymN signaling regulates hSCA beating. (c) SymN activation increases hSCA beating.

Figure 5. hSCAs model myocardial infarction



(a) Hypoxia in hSCAs. (b) Hypoxia induces NE release in hSCAs. (c) Extracellular matrix detected in hSCAs. (d) Increased stiffness under hypoxia. (e) IF shows cell death and fibrosis in hypoxic hSCAs. (f) Blocking NE signaling rescues Ca handling in hypoxic hSCAs.

Conclusion

Here, we developed a simple and flexible strategy to generate symN-innervated cardiac assembloids from hPSCs. The hSCAs are functional, and may be applied to generate more ANS-innervated organoids in the future. Such human-based *in vitro* systems may significantly facilitate the understanding of peripheral network regulation in diseases.

References

1. Wu et al. Efficient Differentiation of Postganglionic Sympathetic Neurons using Human Pluripotent Stem Cells under Feeder-free and Chemically Defined Culture Conditions. *J. Vis. Exp.* (159), e60843, doi:10.3791/60843 (2020).
2. Wu et al. Generation of postganglionic sympathetic neurons using human pluripotent stem cells for electrophysiological and functional assessments. STAR protocol. (2024)