Micropattern-Guided Cardiac Organoid Production for Developmental Toxicity Screening

Plansky Hoang[1,2], Andrew Kowalczewski[1,2], Shiyang Sun[1,2], Jeffrey D. Amack[2,3], Zhen Ma[1,2*]

[1]*Department of Biomedical and Chemical Engineering, Syracuse University,* [2]*BioInspired Syracuse Institute for Material and Living Systems,* [3]*Department of Cell and Developmental Biology, State University of New York Upstate Medical University*

Statement of Purpose: Over the past decade, emerging technologies in stem cell engineering have produced sophisticated organoid platforms by controlling cell fate through material and substrate-based manipulation. Cell micropatterning has been used to integrate biophysical restraint with traditional biochemical factors to study tissue differentiation and morphogenesis. Differentiation of micropatterned human induced pluripotent stem cells (hiPSCs) into cardiac lineage produces region-specific spatially organized tissues with contracting cardiomyocytes surrounded by stromal cells distributed along the pattern perimeter[1]. Using this platform, we generated organoids in various geometries and sizes to investigate the role of biophysical confinement on directing the structural morphology and contractile functions of the cardiac organoids. We applied cardiac organoids as a developmental toxicity screening assay and quantified the embryotoxic potential of nine compounds.

Methods: Non-fouling poly(ethylene glycol) (PEG) was micropatterned via selective etching of the PEG to create circular patterns on 6-well culture plates. hiPSCs were then patterned and differentiated into cardiac lineage, which produced 3D spatial patterned cardiac organoids. We designed and fabricated circles with varying size ranging from 200 - 1000 µm in diameter and assessed how the geometry influences organoid differentiation and spatial organization by comparing the size, structure, and contractile physiology. Using data mining techniques, we correlated structure-function relationships across different sized organoids. Embryotoxicity screening was then performed by supplementing drug compounds during cardiac organoid differentiation.

Results: Micropatterned organoids produced cardiac tissue (cTnT+) at the center surrounded by a monolayer of supportive stomal cells (SM22+) conforming to the shape of the patterns (Fig. 1a). This indicated that biophysical stress supplied by geometrical constraint directs cells in contact with the pattern perimeter to differentiate into the supportive cell type with positive expression of calponin, SM22, smooth muscle actin vimentin. Using data mining of organoid structure and functions, organoids with larger pattern sizes (600 µm, 800 µm and 1000 µm) were better clustered with high consistency, while the organoids from 200 µm and 400 µm patterns showed significant data scattering with separated clusters (Fig. 1b). We observed that the organoids of larger patterns exhibited higher beat rate, while smaller patterns exhibited higher contraction velocities and relaxation velocities. Small organoids were clustered separately, primarily resulting from the significantly prolonged contraction duration correlated with these geometries. From the embryotoxicity screening, we and found that doxylamine succinate (Category A – known safe) treatment had no negative effects, whereas thalidomide (Category X – known toxic) had produced the abnormal cardiac organoids with less cardiac tissue differentiation relative to the untreated controls. More importantly, high concentration of thalidomide (100 µM) impaired the 3D morphology of the cardiac organoids with significantly lower height and width relative to organoids treated with doxylamine succinate and to the controls (Fig. 1c). We also performed parallel *in vivo* studies using live zebrafish embryos. Consistent with the organoid model, exposure with doxylamine succinate (Category A) had negligible effect on the zebrafish embryonic heart development, whereas the effects of thalidomide on zebrafish embryonic heart development were not as significant as what we observed in the organoid model.

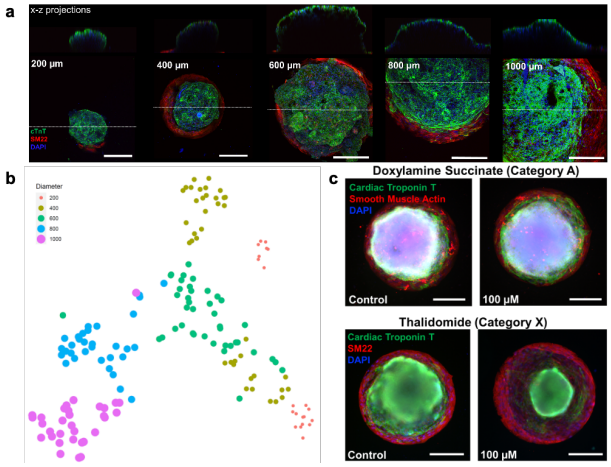

Figure 1. *(a) Confocal images of cardiac organoids generated from varying micropattern diameter. (b) Structure-function relationships derived from data mining of cardiac function and structure parameters. (c) Organoid developmental toxicity assay using known safe and known teratogenic drugs.*

Conclusions: We have demonstrated that cardiac organoid formation is sensitive to biophysical cues and that spatial organization can be controlled by geometric constraint. Cardiac organoids exhibited comparable trends of efficient calcium handling and beating frequency of larger sized organoids. Meanwhile, the contraction duration parameters showed high level of size dependency. The prolongation of contraction duration in cardiac organoids from small patterns is prone to arrhythmia related to the abnormal diastolic functions. Furthermore, we have applied this platform to model heart development and screen for cardiac developmental toxicity. The cardiac organoids exhibited sensitivity and physiological impairment unique to the toxicological properties of the drug. Using this model, we can tailor organoid physiology via geometry and optimize organoid production for advanced and personalized drug screening.

References:1. Ma, Z. Nat. Commun. 2015; 6:7413.