A workflow for nanoscale imaging of cardiomyocytes differentiated from pluripotent stem cells

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Cryo-electron tomography (cryo-ET) is a cryogenic transmission electron microscopy (cryo-TEM) technique that offers high resolution structural analysis of intact macromolecular complexes within their native cell environment. We and others have developed maskless photo-micropatterning methods for functionalizing electron microscopy (EM) grids to facilitate cryo-ET of adherent mammalian cells[1] and to target subcellular regions of interest for imaging[2]. While EM grid micropatterning is a versatile technology for controlling cell shape and position on EM grids, the geometry, composition, incubation time and temperature of the ECM protein must be tailored to each individual cell type and application.

Here we leveraged EM grid micropatterning to direct cell shape and positioning of human induced pluripotent stem cell cardiomyocytes (hiPSC-CM) for nanoscale imaging with cryo-ET. We cultured the cells on EM grids with rectangular Matrigel islands that diagonally span each grid-square to maximize the cell area available to the e-beam (See Fig. 1). Based on prior studies, we chose an aspect ratio of 1:7 for the micropatterns to promote a more mature hiPSC-CM phenotype.[3] We introduced a post-micropatterning sterilization step to prevent contamination during the week-long culture of the cells on the grids and vitrified the cells using established methods for plunge freezing. The cells assembled on imageable locations of the EM grids and we could distinguish the locations of nuclei and cell-cell contacts in the vitrified cells using cryo-scanning electron microscopy (cryo-SEM) (Fig. 2). Because hiPSC-CMs are too thick to be imaged directly with cryo-TEM, we refined conditions to mill thin (<200 nm) lamella using cryo-focused ion beam milling (cryo-FIB)(Fig. 3). We then successfully collected cryo-ET tilt series data at several locations along each lamella. Our reconstructed cryo-tomograms revealed a diversity of sub-cellular structures in cardiomyocytes, such as intercalated disks, sarcoplasm reticulum, sarcomere, granules, and ribosomes (Fig. 4). In the future we will combine our workflow for cryo-FIB and cryo-ET of hiPSC-CMs with subtomogram averaging to resolve the 3D organization of hiPSC-CMs in molecular scale detail and compare architectural differences between normal hiPSC-CM and those bearing disease-causing mutations to shed light on the mechanistic underpinnings of this class of prevalent genetic disorders.

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[1] Engel, L., et al. (2019). *Journal of Micromechanics and Microengineering*, *29*(11), 115018.
[2] Engel, L., et al. (2021). *Journal of structural biology*, *213*(4), 107791.
[3] Ribeiro, A.J., et al. (2015). *PNAS*, *112*(41), 12705-12710.

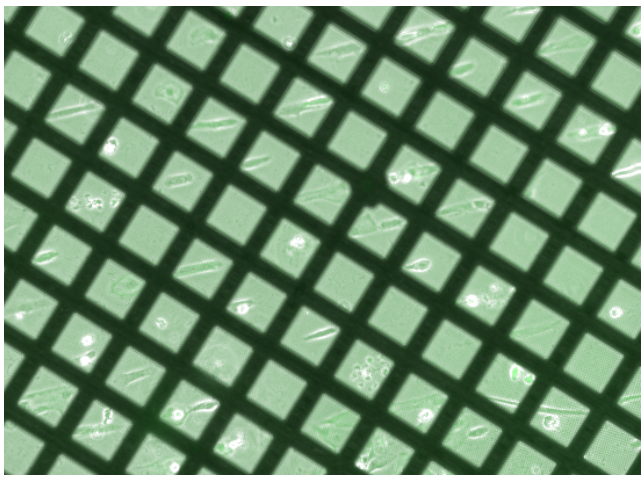

Figure 1: Cells adopt elongated shapes on Matrigel micropatterns. Live cells assembled on micropatterned 200 mesh EM grids prior to vitrification.

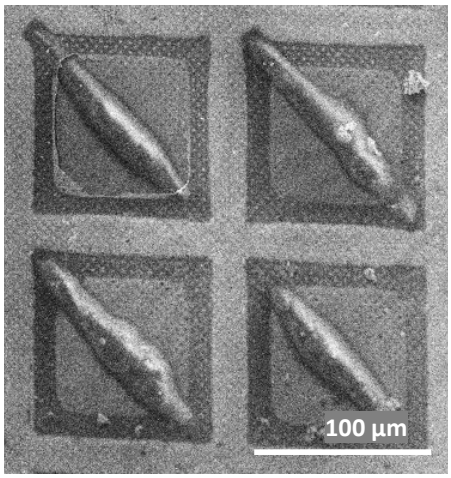

Figure 2: Vitrified cells on micropatterned grid. Cryo-SEM of cells assembled on micropatterned EM grid.

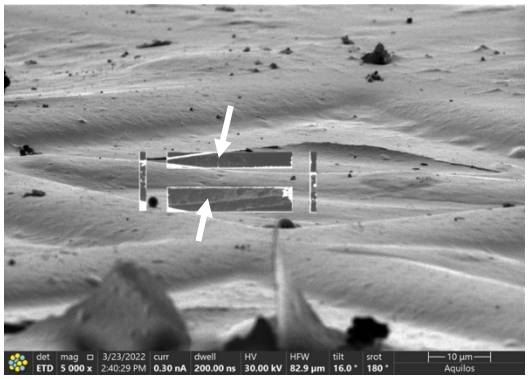

Figure 3: Cryo-focused ion beam milling was used to prepare thin lamella for cryo-TEM. Micrograph was produced using a gallium ion beam during the cryo-FIB milling process. White arrows point to a region of interest for imaging: the meeting between two cells.

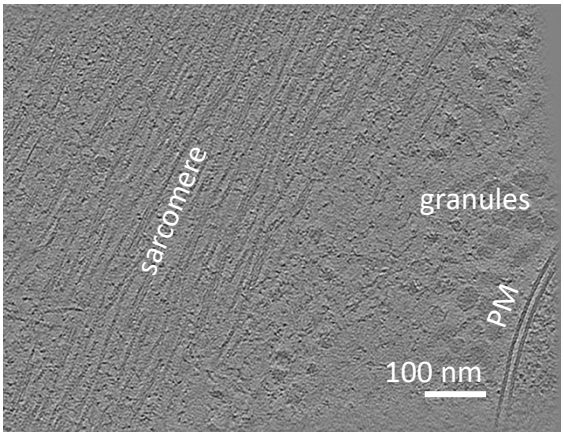

Figure 4: Slice of reconstructed cryo-tomogram shows intact nanoscale subcellular structures. Sarcomere can be seen on the left side. Plasma membranes (PM) indicating a cell-cell contact can be seen on the bottom right.