Micro- and Nanofabrication Technologies for Cellular Cryo-electron Tomography Studies

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Cryo-electron tomography (cryo-ET) is a transmission electron microscopy (TEM) technique that provides unparalleled three-dimensional visualization of complex biological architectures within intact, hydrated cells. Integration of micro- and nanotechnologies into cryo-ET sample preparation pipelines, have dramatically contributed to the field in recent years. A key example is cryo-focused ion beam (cryo-FIB) milling, which enables the preparation of thin lamellae from vitrified samples, expanding the range of samples that can be studied with cryo-ET to include cells and tissues that would otherwise be too thick to image with TEM (>500 nm). We previously developed a micropatterning technology using maskless photolithography to functionalize TEM supports so that they can position and shape cells at high spatial accuracy, streamlining cryo-FIB and cryo-ET sample preparation[1]. We demonstrated the utility of this technique for structural studies of lymphocytes[2], cardiomyocytes[3], and endothelial cells[4]. While micropatterning can recapitulate certain aspects of the cell microenvironment (e.g., spatial constraints from neighboring cells), it cannot recapitulate the topography of the extracellular matrix (ECM), a key aspect of the cell microenvironment known to influence cellular function.

Here we present a new class of nanopatterned TEM grids, customized for cryo-ET, that can provide cells with programmed topographical cues that better mimic an in vivo environment (Figure 1). We produced ECM nanofibers using electrospinning and aligned them directly onto TEM grids using a 20 cm rotating collection disk. The sharp edge of the disc served to focus the electrical field and direct the polymer jet onto the grids that were affixed to it. The tangential velocity of the disc was varied to alter the degree of fiber alignment on the grids to generate aligned (healthy mimicking) and randomly oriented (unhealthy) ECM conditions. We analyzed the fiber morphology, orientation, and porosity using high resolution scanning electron microscopy (Figure 2). We demonstrated that our engineered ECM constructs are compatible with endothelial cell culture and vitrification by plunge freezing. This platform facilitated cryo-ET of the interior of endothelial cells cultured on thin films with different nanotopographies. Our reconstructed cryo-tomograms show nanoscale features such as mitochondria, granules, ribosomes, and vesicles within cells. In the future, this platform can be used to study the nanoscale underpinnings of cellular sensitivity to substrate nanotopography as well as the downstream effects of disruptions to ECM organization on cell ultrastructure.

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[1] Engel, L., et al. (2019). *Journal of Micromechanics and Microengineering*, *29*(11), 115018.
[2] Engel, L., et al. (2023) *bioRxiv* and under review 2023.08.05.552110
[3] Woldeyes, R., et al. (2023) *bioRxiv* and under review 2023.10.26.564098
[4] Engel, L., et al. (2021). *Journal of structural biology*, *213*(4), 107791.

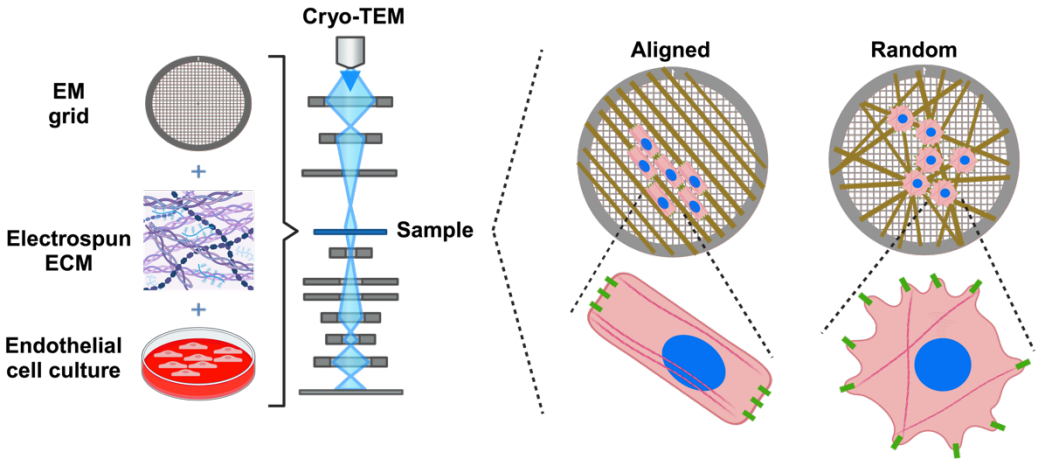

Figure 1: Schematic depicts a platform for high resolution cryo-TEM imaging of cells grown on ECM with different topographical cues.

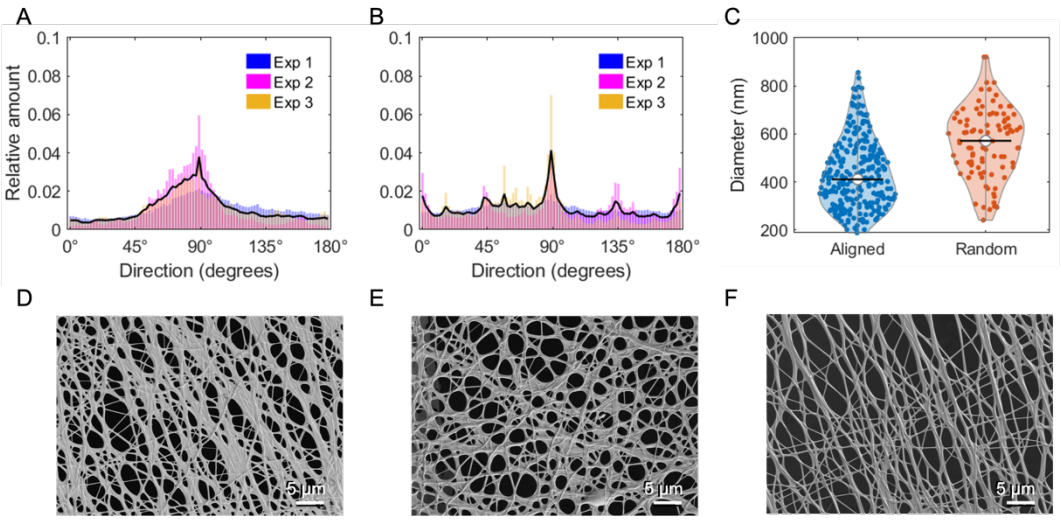

Figure 2: Fiber characterization. (A-B) Histograms of preferred fiber orientation for three separate experiments for aligned (A) and random (B) fiber orientations. The black line represents a weighted average for the three experiments for each condition. (C) Violin plot depicts fiber diameter for random (red) and aligned (blue) oriented fibers. The black line indicates the median. (D-F) HRSEM images of gelatin fibers electrospun from 28% solution directly onto gold TEM grids. (D) Aligned fibers. (E) Randomly oriented fibers. (F) Aligned oriented fibers after four months of storage in a desiccator.