

Engineering Liver Extracellular Matrix Nanofibers to Functionally Mature iPSC-derived Liver Cells

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Introduction: Drug-induced liver injury (DILI) remains a leading cause of drug attrition and acute liver failures, partly due to the inadequacy of animal models to accurately predict human clinical outcomes, which necessitates the utilization of in vitro models of the human liver. However, primary human hepatocytes (PHHs) are in short supply for routine drug screening. In contrast, induced pluripotent stem cells (iPSCs)-derived hepatocyte-like cells (HLCs) are a nearly unlimited cell source but display a fetal-like (versus adult-like) phenotype when differentiated using conventional protocols on tissue culture plastic or glass adsorbed with 2D extracellular matrix (ECM) proteins. Electrospinning can produce porous nanoscale 3D fibers that have a large surface area and present a high density of receptor ligands to modulate cell phenotype. However, the application of electrospinning to generate 3D liver-derived ECM substrates for HLC differentiation remains unexplored. Therefore, here we developed methods to a) electrospin nanofibers of different porosities and diameters using porcine liver ECM (PLECM) with or without type I collagen and b) use these fibers to determine functional modulation in iPSC-derived HLCs while using PHHs as a control cell type relative to conventional adsorbed ECM substrates.

Materials and Methods: ECM dissolved in optimized solvents was electrospun from a high voltage DC power supply and collected onto oxidized glass cover slides. To generate adsorbed ECM coated glass controls, glass slides were treated with oxygen plasma and then coated with 100 $\mu\text{g/ml}$ collagen and/or PLECM for 2 hours. Cryopreserved PHHs were seeded on the nanofibers at the density of 200K cells/well in 24 well plates. iPSCs were differentiated into HLCs over 20 days using a published protocol that uses specific growth factor cocktails (e.g., Activin A, bFGF, BMP4, HGF, OSM) to differentiate iPSCs adhered onto tissue culture plastic into definitive endoderm, specified hepatic endoderm, hepatoblasts, and finally immature HLCs. Alternatively, iPSCs differentiated into definitive endoderm were lifted from the plate using optimized dissociation protocols, seeded at 300K cells/well onto various nanofiber configurations, and then further matured over 20 days using a similar protocol as above. The hepatic phenotype was assessed using published assays.

Results and Discussion: The PLECM/collagen mixture formed nanofibers with a diameter of 192 ± 83 nm (Fig. 1A). PHHs were cultured on nanofibers and adsorbed ECM glass controls for 3 weeks. Albumin secretion was ~4-fold higher on the nanofibers than the adsorbed ECM glass control (Fig. 1B, C). Next, iPSC-derived definitive endodermal cells (90% positive for SOX17, an endodermal marker) were seeded onto nanofibers and further matured for 20 more days (Fig. 1D, E). Most cells had alpha-fetal-protein (AFP) expression, while ~50% of the HLCs expressed albumin, a marker of mature hepatocytes (Fig. 1E). After 20 days of differentiation, HLCs differentiated onto the PLECM/collagen nanofibers had ~5-fold higher albumin secretion than the adsorbed ECM glass control (Fig. 1F).

Conclusion: We showed for the first time that electrospun nanofibers derived from liver ECM can induce significantly higher functions in PHHs and iPSC-derived HLCs. Future efforts are focused on testing other compositions of nanofibers on the HLCs, characterizing additional liver pathways, including liver non-parenchymal cells, and testing the platform for predicting human DILI.

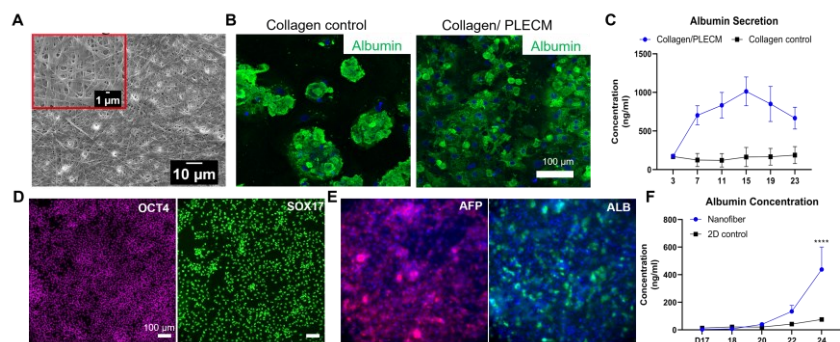


Figure 1. (A) Scanning electron microscopy images of crosslinked nanofibers. (B) Immunofluorescent images of PHHs on plastic control (left) or collagen/PLECM nanofiber (right). (C) Albumin secretion from PHHs on adsorbed collagen glass control or collagen/PLECM nanofibers. (D) Immunofluorescent images of stage-specific markers in iPSC (left) and iPSC-derived definitive endoderm (right). (E) Immunofluorescent images of HLCs on collagen/PLECM nanofibers on differentiation day 25. (F) Albumin secretion of HLCs on nanofiber vs glass control.