Development of a functional and perfusible hPSC-derived cholangiocyte duct for biliary disease modeling

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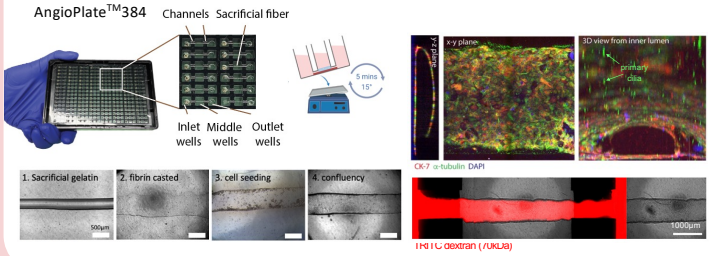
Introduction

There are currently no reliable in vitro models to study the pathological mechanisms of biliary disease progression underlaying chronic biliary diseases such as cystic-fibrosis related liver disease (CFLD) and primary sclerosing cholangitis (PSC). Conventional monolayer and 3D organoid systems do not include fluid flow or bile acids, which are crucial physiological signals impacting cholangiocyte function. Here, we modified our published protocol in generating highly functional human pluripotent stem cell (hPSC) derived cholangiocytes to adapt into the ***AngioPlate™384*** platform for constructing a perfusible 3D bile duct. This allows us to monitor the effect of physiological versus toxic levels of bile acids on epithelium integrity, as well as to monitor stromal cell interaction with cholangiocytes under physiological fluid flow to study biliary fibrosis.

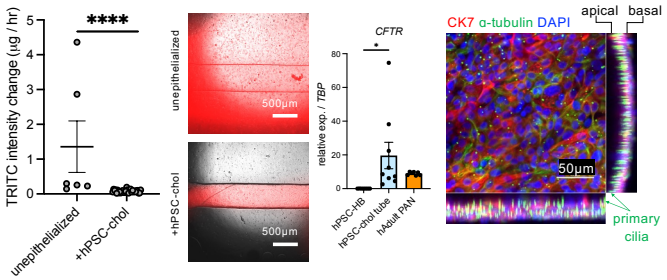
Perfusible 3D bile duct in AngioPlate™384

Cholangiocytes can be differentiated within the AngioPlate™ from hPSC-derived hepatic progenitor cells. The bile duct is perfusible, as indicated by TRITC dextran passage through the tube. This unique platform enables cholestatic and fibrotic biliary disease modeling by assessing epithelial barrier integrity in presence of **physiological fluid flow** and **bile acids (BAs)**.



hPSC-cholangiocyte bile duct maturation

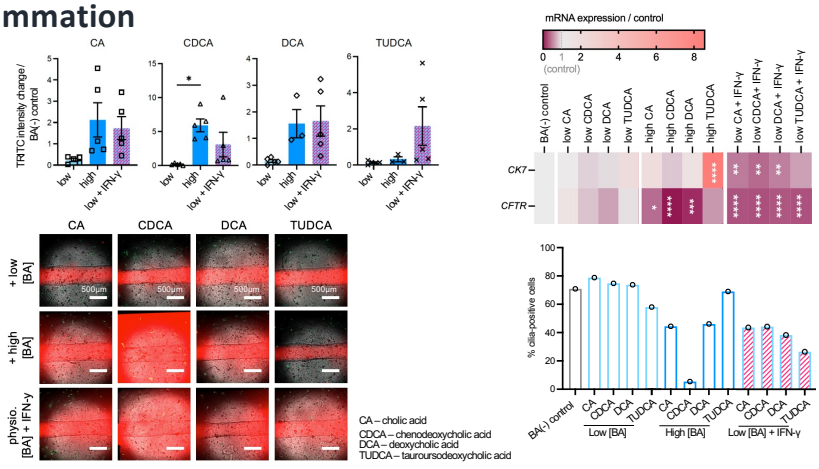
Mature intrahepatic cholangiocytes maintain a tight epithelial barrier to modulate bile flow and prevent the leakage of detergent-like bile. They express higher level of cystic fibrosis transmembrane conductance regulator (CFTR) and display primary cilia at the apical surface.



Modeling biliary cholestasis and inflammation

Cholestasis refers to toxic bile acid build up to a toxic level within the biliary network. Chronic BA accumulation damages the cholangiocyte epithelial barrier, compromises cholangiocyte function and damages surrounding tissue. We treated hPSC-chol ducts with low or high concentrations of various BAs. Compared to low levels of BAs, higher concentrations compromised the barrier integrity and reduced the expression of functional gene markers.

To monitor inflammatory disease progression, specifically the effect of inflammatory cytokine presence in bile flow on cholangiocyte function, we added interferon-gamma (IFN-γ) together with low BA concentrations to hPSC-chol tubes. Interestingly, the presence of IFN-γ on top of low concentration of BAs disrupted the hPSC-chol epithelium, leading to barrier leakage and functional marker reduction.

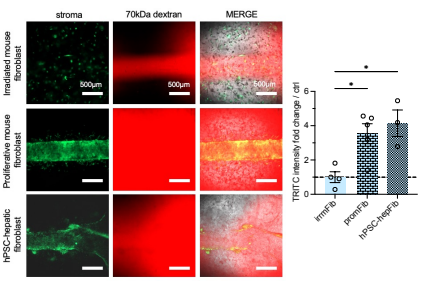


Conclusion

We developed a perfusible bile duct from hPSC-derived cholangiocytes in the ***AngioPlate™384.*** We successfully assessed hPSC-chol epithelial barrier integrity and functional marker expression in presence of bile acids and a pro-inflammatory cytokine. We also modeled fibrosis by co-culturing proliferative stromal cells with the hPSC-chol duct.

Biliary fibrosis modeling

Focal biliary fibrosis, which occurs concentrically around bile ducts, is characteristic of chronic biliary diseases such as CFLD and PSC. To model the fibrotic event, we introduced a proliferative mouse stromal cell or hPSC-derived hepatic mesenchymal cells, both of which proliferated and wrapped around the tube, causing increased barrier leakiness.



References

Ogawa, M., Jiang, J. X., Xia, S., Yang, D., Ding, A., Laselva, O., Hernandez, M., Cui, C., Higuchi, Y., Suemizu, H., Dorrell, C., Grompe, M., Bear, C. E., & Ogawa, S. (2021). Generation of functional ciliated cholangiocytes from human pluripotent stem cells. *Nature communications*, *12*(1), 6504.

Rajasekar, S., Lin, D. S. Y., Zhang, F., Sotra, A., Boshart, A., Clotet-Freixas, S., Liu, A., Hirota, J. A., Ogawa, S., Konvalinka, A., & Zhang, B. (2022). Subtractive manufacturing with swelling induced stochastic folding of sacrificial materials for fabricating complex perfusable tissues in multi-well plates. *Lab on a chip*, *22*(10), 1929–1942.

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