Optimisation of 3D iPSC organoid cultures for enhanced hepatocyte differentiation

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Aim

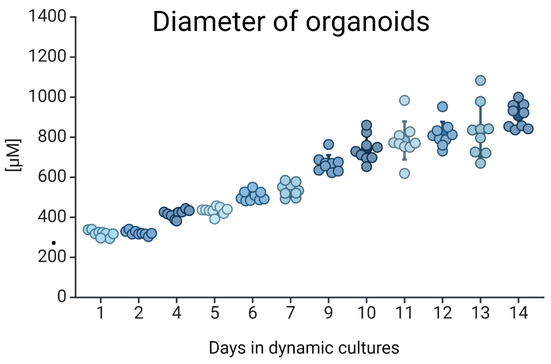
(1) Develop accessible methods for producing iPSC-based hepatic organoids.
(2) Apply StressFree 3D technology to simplify organoid differentiation.

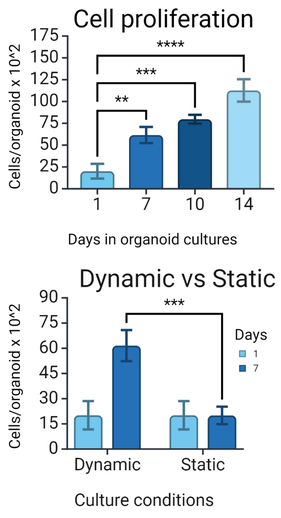
Methods

(1) Takara Bio Cellartis iPS Cell to Hepatocyte Differentiation System
(2) StressFree 3D in the ClinoStar® System utilising microgravity ClinoReactors
(3) qPCR, immune staining, ATP test, planimetry, proteomics (LC-MS/MS)

Enhanced iPSC Proliferation and Viability

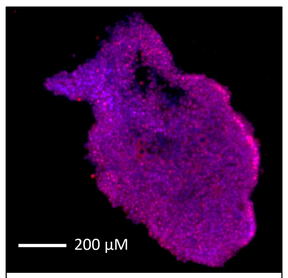
iPSCs demonstrate superior proliferation rates when cultured in the ClinoStar® system (dynamic) compared to the low-binding plate (static) method. iPSC cells remain pluripotent in 3D cultures for a minimum of 2 weeks.



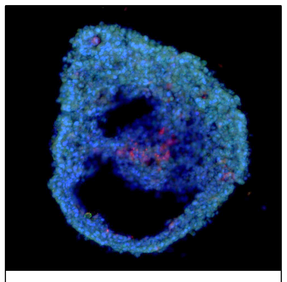


The viability of cells in all organoids was 95% (median) with a coefficient of variation (CV) of 4%.

iPSC cells cultured for 14 days.



Ki67 - red, DAPI - blue



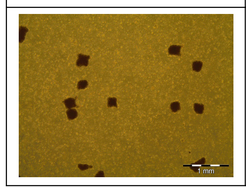
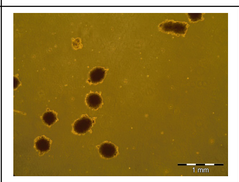
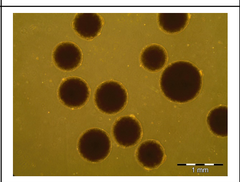
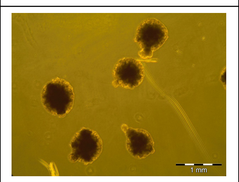
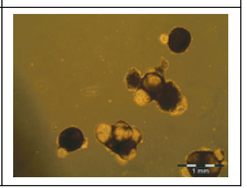
OCT4 - green, SSE1 – red, DAPI - blue

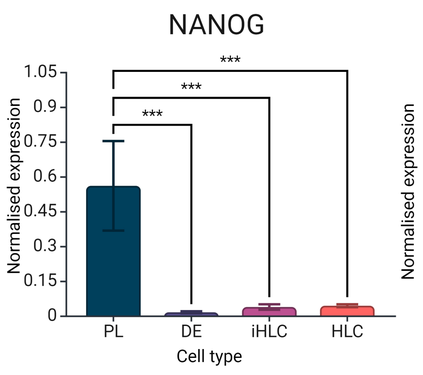
Background

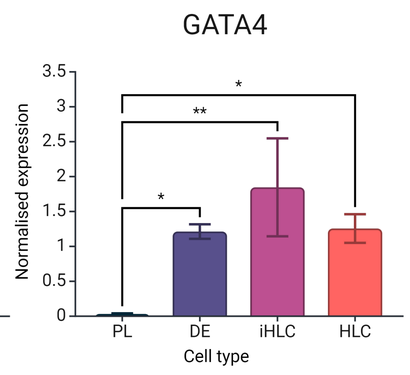
A human-specific model accurately representing hepatic physiology is critical for improving the predictive power of drug toxicity testing. Current models often fail to recapitulate the complexity of human liver tissue, leading to a demand for advanced methods such as iPSC-based hepatic organoids.

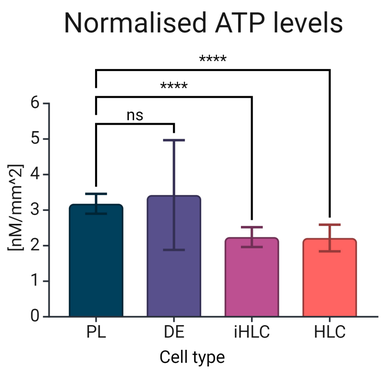
In Situ 3D Differentiation of iPSC to Hepatocyte-like Cells

Direct differentiation within the organoids maintained in the ClinoStar® system efficiently forms definitive endoderm (DE), immature hepatocyte-like (iHCL), and hepatocyte-like cells (HLC).

Day 1. Pluripotent (PL)	Day 5. Pluripotent (PL)	Day 9. Definitive Endoderm (DE)	Day 19. Immature Hepatocyte-like (iHCL)	Day 26. Hepatocyte-like (HLC)
				



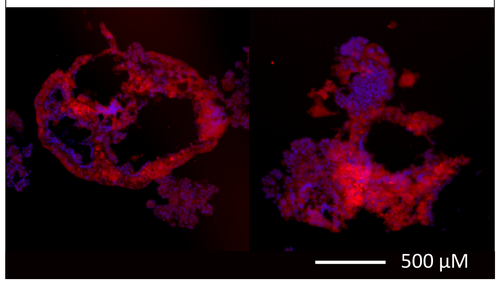


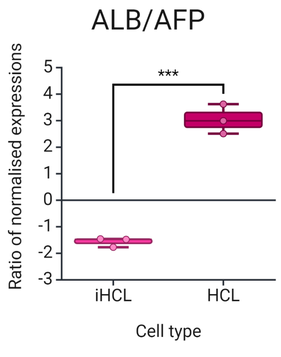


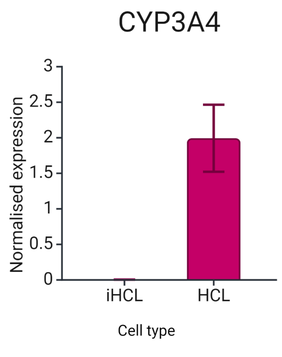
Maturation of Hepatic Organoids

Organoids exhibit increased maturity over time, as evidenced by rising albumin to alpha-fetoprotein mRNA ratios and the expression of CYP3A4, a crucial enzyme involved in drug metabolism.

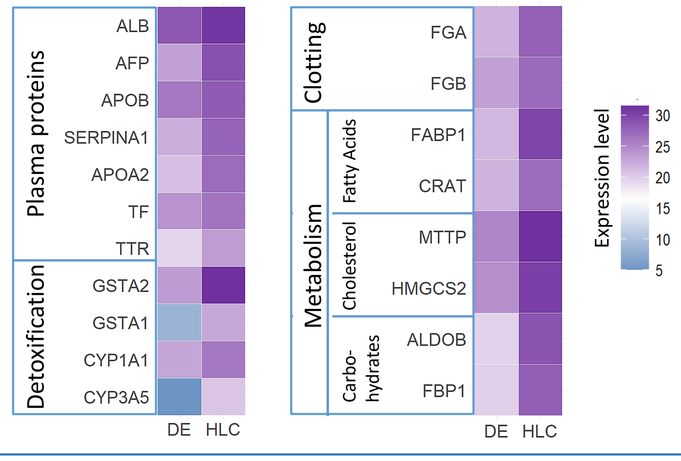
Albumin Expression







Proteomics Confirms Progressive Maturation of Hepatic Phenotype



The progressive increase in plasma and clotting proteins, detoxification and metabolic enzymes highlights these organoids' potential for drug toxicity testing and disease modelling.

Conclusions

Direct differentiation of iPSCs in spheroids maintained in the ClinoStar® system supports the creation of viable, mature hepatic organoids. This simplified protocol offers a promising drug testing and disease modelling platform.

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