Generation and characterisation of hepatocyte like cell derived from healthy human induced pluripotent stem cell: applications in translational research

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BACKGROUND

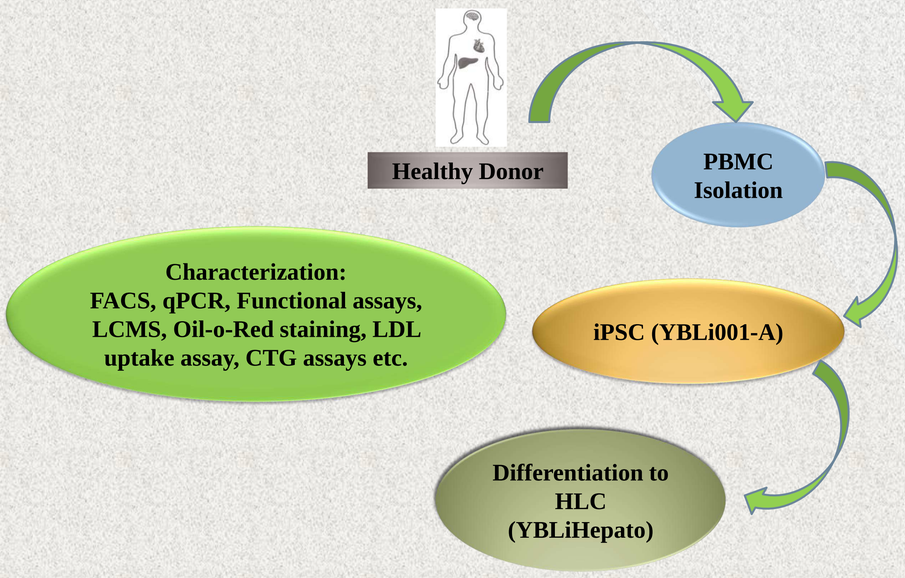
- Liver disease stands as a foremost contributor to mortality in globally.
- Over time, investigations into liver disease have faced inhibitions, primarily due to the limited availability of donors.
- Furthermore, the inadequacies of animal models in fully replicating human liver function have posed additional challenges.
- The in vitro cultivation of hepatocytes has proven complicated due to the rapid de-differentiation of primary hepatocytes in culture conditions.
- This prompts us to identify an alternative option for the generation of hepatocytes.
- It is known that upon induction, human induced pluripotent stem cells (hiPSCs) have potential to differentiate into Hepatocyte-Like Cells (HLCs).

OBJECTIVES

The objective of the studies:

- To generate a robust method for the generation of cost-effective Hepatocyte Like Cells from hiPSCs in compliance with good manufacturing practice (GMP) guidelines.

METHODS



RESULTS

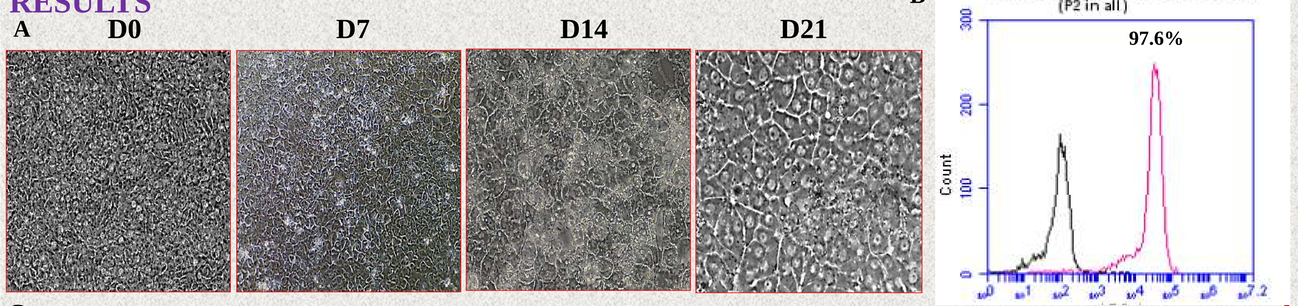


Fig. 1: YBLiHepato: demonstrate hepatocyte-like cells morphology (A) and positive expression of hepatic marker: FACS (B) & (C) qRT-PCR.

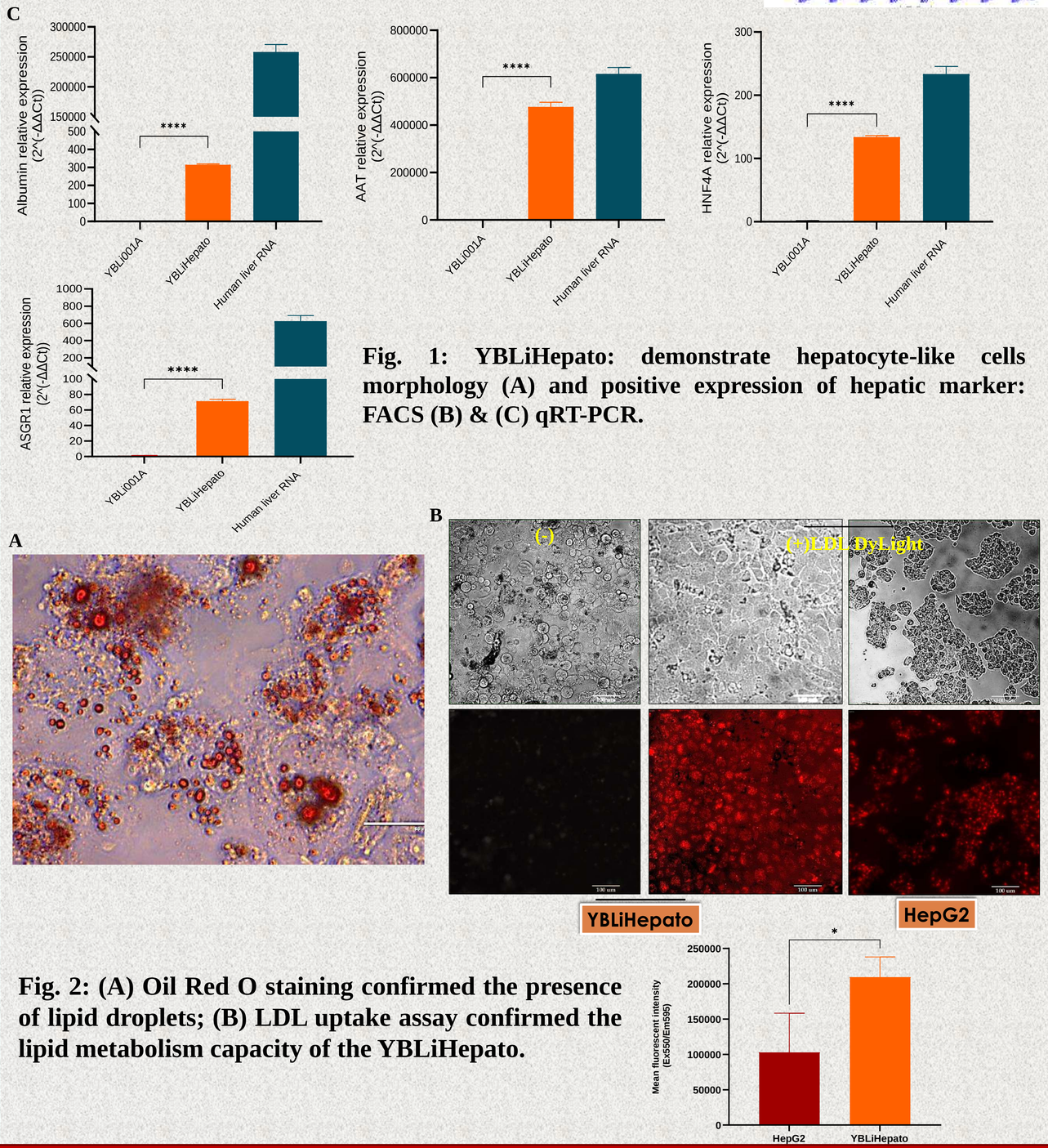
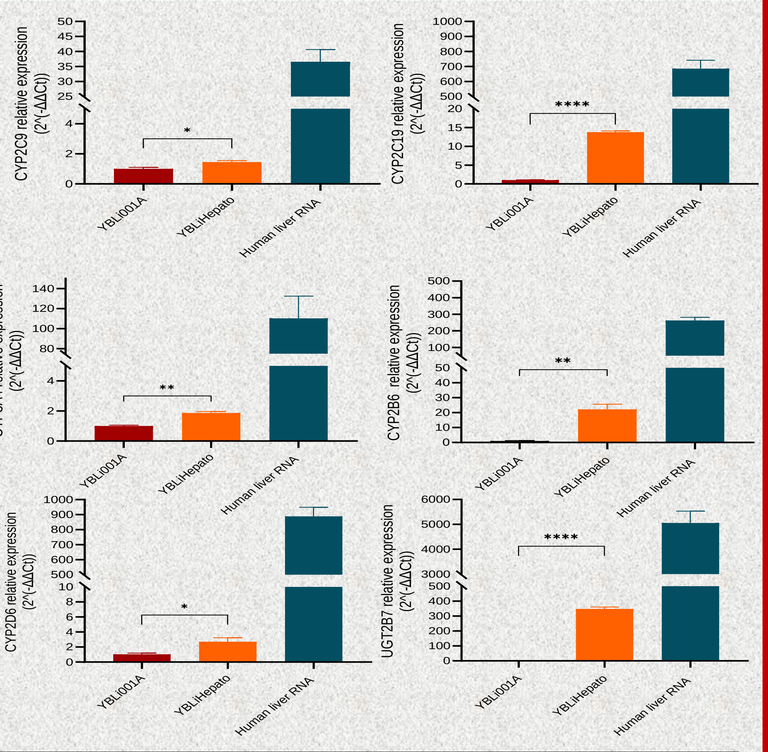


Fig. 2: (A) Oil Red O staining confirmed the presence of lipid droplets; (B) LDL uptake assay confirmed the lipid metabolism capacity of the YBLiHepato.

Fig. 3: qRT-PCR studies confirms the presence of for CYP gene expression in YBLiHepato



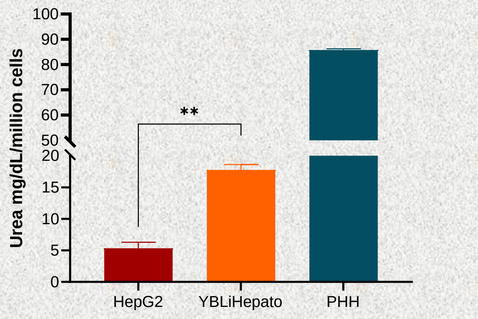


Fig. 4: YBLiHepato have active urea production capacity.

A

Cyp Activity				
CYP Isoforms	Probe substrate	Final Substrate Conc.(µM)	Incubation Time (Minutes)	Marker metabolite
CYPlA2	Phenacetin	10	120	Acetaminophen
CYP2B6	Bupropion	10	120	Hydroxybupropion
CYP2C9	Diclofcnac	10	120	4′-Hydroxydiclofenac
CYP2C19	S-Mephenytoin	10	120	4 -Hydroxymephenytoin
CYP2D6	Dextromethorphan	10	120	Dextrorphan
CYP3A4	Testosterone	10	120	6β-Hydroxytestosterone

B

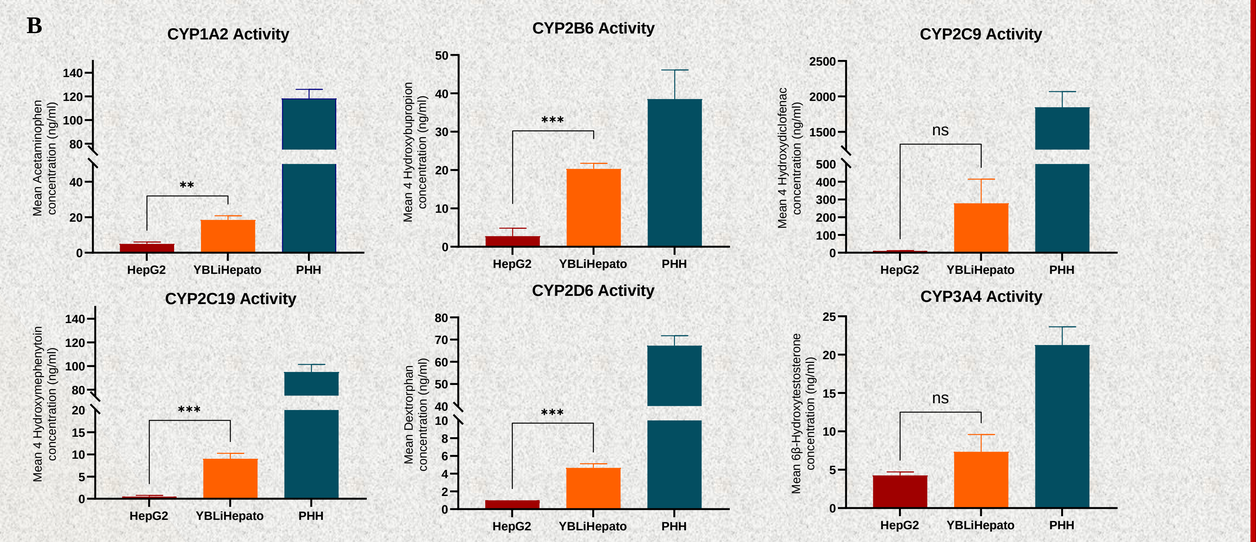


Fig. 5: LC-MS/MS studies confirmed the activity of major CYP isoforms in YBLiHepato. The cells incubated with probe substrate and metabolite concentration was determined.

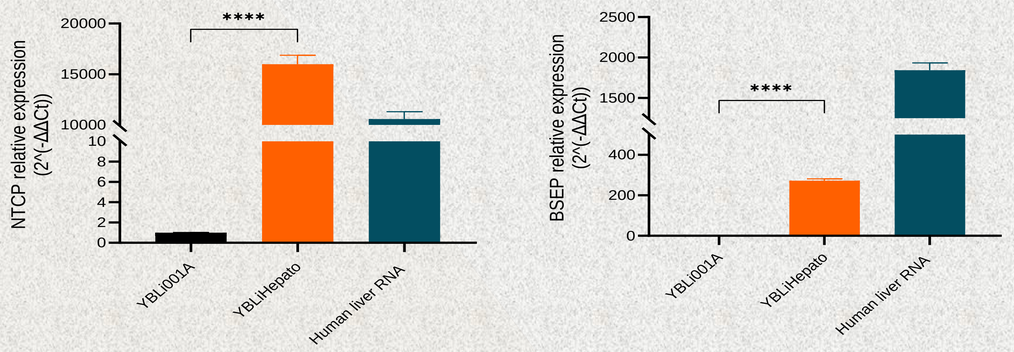


Fig. 6: qRT-PCR analysis of YBLiHepato confirmed the presence gene expression of Bile Transporters.

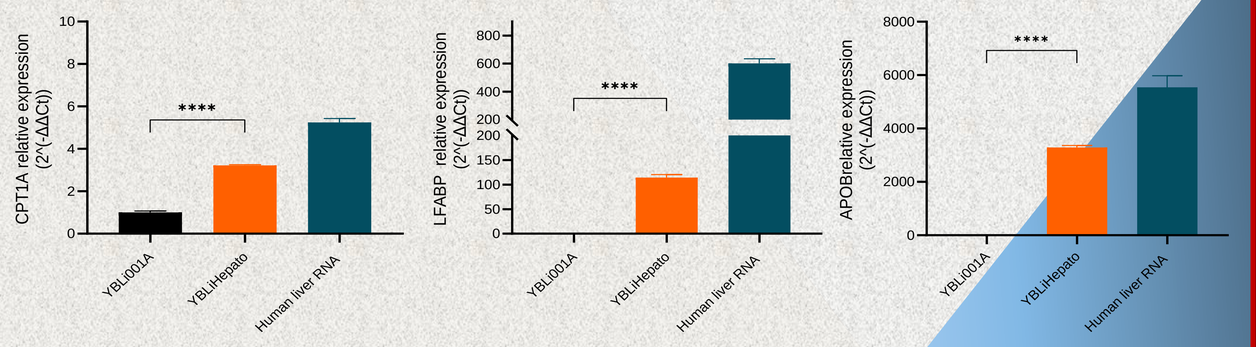


Fig. 7: qRT-PCR analysis of YBLiHepato confirmed the presence gene expression of Fatty Acid Metabolism genes

CONCLUSION AND FUTURE DIRECTION

- We have sequentially generated definitive endoderm (DE), hepatic progenitor cells, and finally fully functional HLCs from hiPSCs, which has been propose to address the shortage of human hepatocytes for hepatotoxicity, drug metabolism, and hepatic research.

ACKNOWLEDGEMENT

The authors would like to thank the entire Yashraj team for providing scientific inputs and constructive critique during the course of this project.