Bioprocess Engineering of a Human 3D Cell Model for Primary Hyperoxaluria type 1

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BACKGROUND

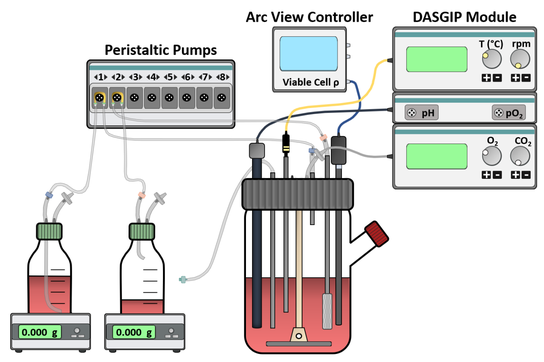
Primary hyperoxaluria type 1 (PH1) is a rare metabolic disorder caused by genetic mutations in the hepatic alanine-glyoxylate aminotransferase (AGT). Deficient AGT results in an excessive oxalate production by the hepatocytes in the liver that leads to the accumulation of calcium oxalate crystals in the kidney, resulting in several kidney complications. The combined liver-kidney transplantation is currently the only curative treatment, but high risks and short supply of organs strongly supports the development of, more advanced cell and/or gene therapies as well as disease models that accurately recapitulate PH1 pathophysiology. Therefore, the development of in vitro models from hiPSC (human induced pluripotent stem cells) derived from patients could be a useful tool to test the therapeutic efficacy of these new treatments.

AIM

Improve the 3D bioprocess for hepatic differentiation of hiPSC to generate high number of functional hepatocytes-like cells for PH1 disease modelling.

EXPERIMENTAL DESIGN

Culture system

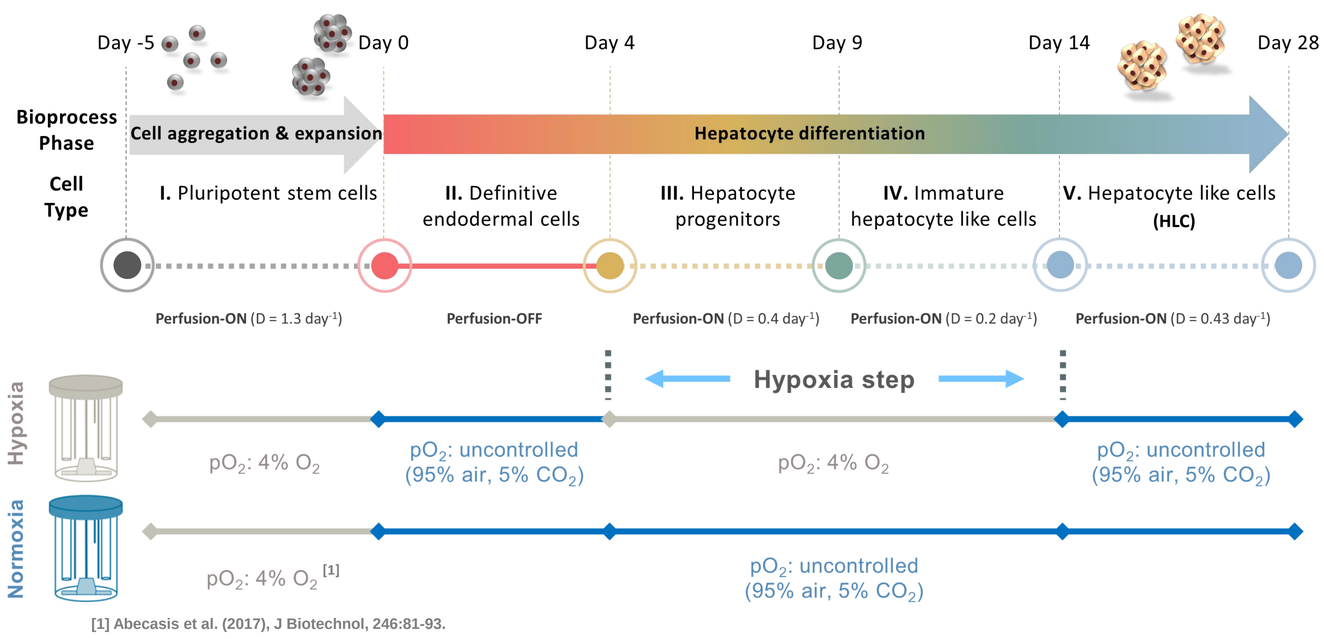


Stirred tank bioreactors (DasGip cellferm-pro system, Eppendorf AG), operated in perfusion (**WV- 200mL**);

Culture media and supplements for hiPSC expansion (Cellartis®DEF-CS Xeno-Free 3D) and differentiation were developed by Takara Bio Europe AB;

Online monitoring of: pO_2, pH, temperature and **permittivity (dielectric spectroscopy);**

Integrated bioprocess for hiPSC 3D expansion and hepatic differentiation



[1] Abecasis et al. (2017), J Biotechnol, 246:81-93.

hiPSC lines used:

- **PH1.hiPSC** (disease cell line);
- **ChiPSC18.hiPSC** (healthy-cell line/control cell line).

Monitoring of:

- Viable cell concentration;
- Aggregate concentration/size;
- Gene and protein expression;
- Hepatic function;

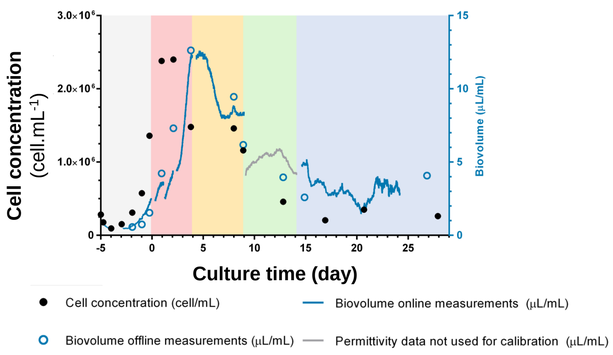
Dissolved oxygen control:

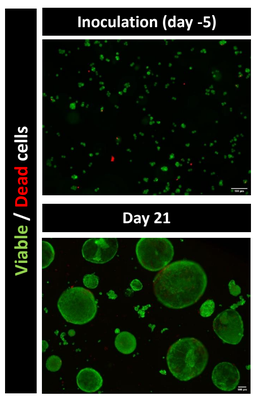
Select the most fitting hepatic differentiation strategy:

- **Hypoxia** (pO_2 :4% O_2 between day 4 and day 14);
- **Normoxia** (pO_2: uncontrolled 95% air, 5% CO_2);

RESULTS

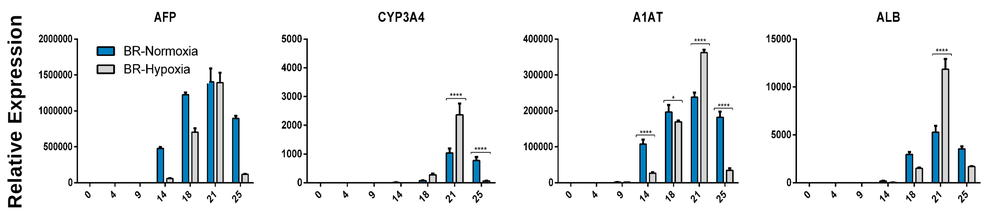
1 Online monitoring of PH1.hiPSC expansion and hepatic differentiation[2]



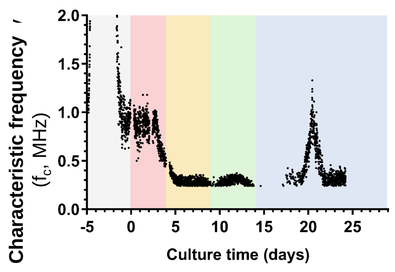


$$Biovolume = (Aggregate\ concentration) \times \frac{4}{3}\pi(Aggregate\ mean\ radius)^3$$

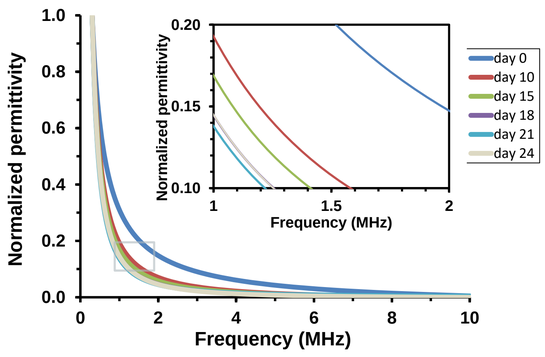
Cell permittivity (Δε) measured by dielectric spectroscopy follows total aggregate biovolume and does not correlate well with viable or total cell concentration. Viable cell concentration increases faster than total aggregate biovolume (**delayed response**).



Hepatic maturation markers expression higher at **day 21** and in **BR-Hypoxia**



Characteristic frequency (f_c) estimated by the probe shows a **clear peak around day 21**.

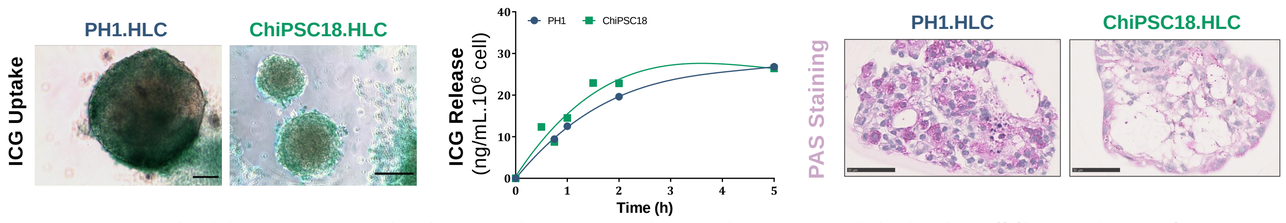


β-dispersion peaks at **day 21** and corresponds to highest observed value in hepatic biomarkers

β-dispersion curve could be used as an indicator of **differentiation** progression

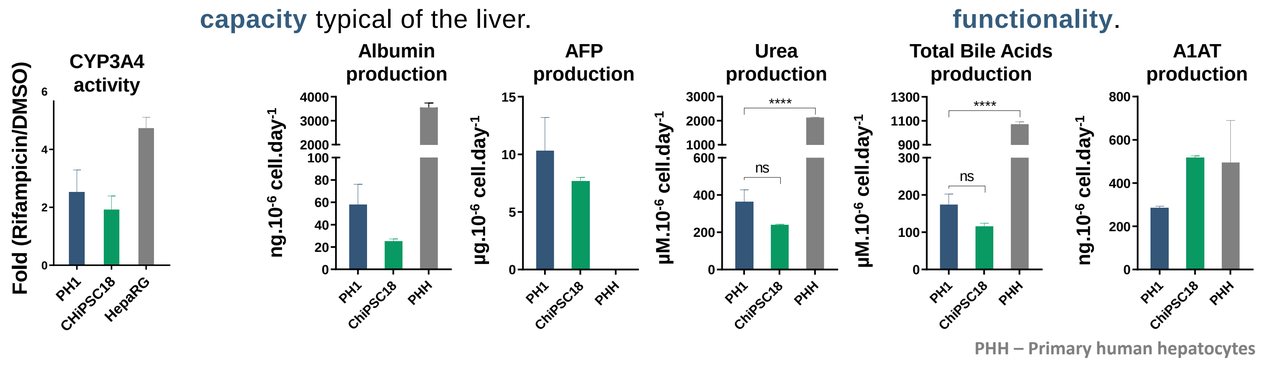
[2] Isidro, I. A et al, (2021), Biotechnology and Bioengineering, 1–8.

2 HLC derived from disease (PH1) and healthy (ChiPSC18) cell lines show similar hepatic function at day 21



PH1.HLC and **ChiPSC18.HLC** had **capacity to uptake and release** Cardiogreen drug, showing **drug metabolization capacity** typical of the liver.

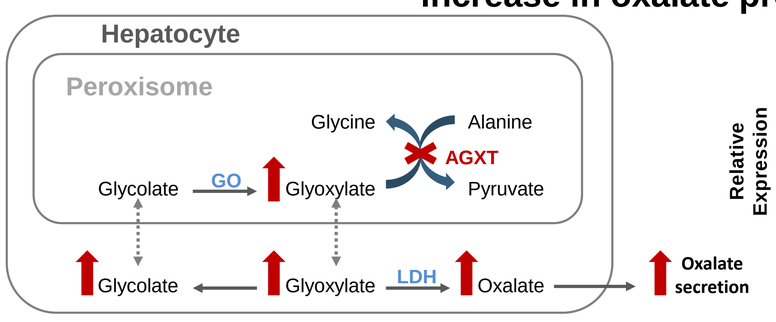
HLC in both cell lines **show glycogen storage** (PAS) **suggesting hepatic functionality**.

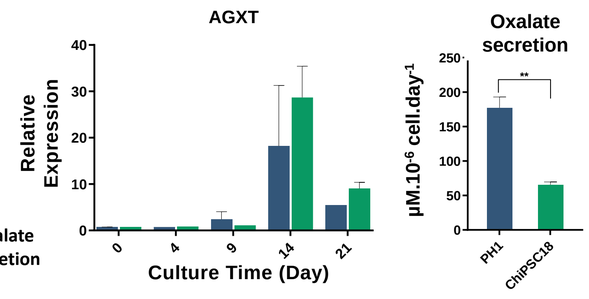


PHH – Primary human hepatocytes

HLC generated show functional features of human liver with CYP3A4 enzyme activity and the capacity to synthesize urea, hepatic proteins (AFP, A1AT, ALB) and bile acids.

3 HLC derived from disease (PH1) cell line show an increase in oxalate production





PH1.HLC showed similar gene expression profile of AGXT but a **higher oxalate production** compared with the **ChiPSC18.hiPSC** suggesting a deficient AGT folding and/or activity, resembling the PH1 phenotype disease *in vivo*.

CONCLUSIONS

- **Hepatic differentiation was improved** with hypoxia stimulus generating functional HLC;
- Dielectric spectroscopy is a promising tool to monitor expansion and differentiation process of hiPSC aggregates in STB;
- HLC derived from **PH1.hiPSC showed** a significant **increase in oxalate production** compared with the HLC derived from **ChiPSC18.hiPSC mimicking the disease phenotype.**

ACKNOWLEDGEMENTS

This work was carried out within the scope of the project ERAdicatPH (Ref. E-Rare3/0002/2015) funded by the European ERA-Net for Research Programmers on Rare Diseases. "iNOVA4Health – UIDB/04462/2020 and UIDP/04462/2020, a program financially supported by Fundação para a Ciência e Tecnologia / Ministério da Ciência, Tecnologia e Ensino Superior, through national funds is acknowledged" "Funding from INTERFACE Programme, through the Innovation, Technology and Circular Economy Fund (FITEC), is gratefully acknowledged ".