Functional improvement of iPS cell-derived Hepatocyte (ReproHepato™) by utilizing 3D culture system

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Introduction

Peripheral blood provides easy access to adult human cell types for reprogramming purposes. In late 2012, two groups demonstrated the effective isolation, expansion, and subsequent generation of retrovirally-induced iPS cell lines from endothelial progenitor cells (EPCs) derived from human primary hepatocytes have been utilized for high-throughput screening in early stages of drug discovery to evaluate thousands of potential therapeutic compounds. Yet, considerable lot-to-lot variability exists in commercially available cells since specific lots originate from individual (genetically unique) donors. Furthermore, human primary hepatocytes have primary hepatocytes have cell supply and cell expansion limitations from single donors. To overcome these disadvantages, human induced pluripotent stem (IPS) cells have attracted attention because they can be clonally expanded from the same donor and differentiated into hepatocytes in large quantities. Nevertheless, cell immaturity and donor variation are common drawbacks posed by IPS cell-derived hepatocytes. In order to address these issues, we have utilized 3D cultivation for maturating hepatocytes of IPS cell-derived (clonal) hepatocytes to average out the variation issue between donors.

We have demonstrated increased maturation of human IPS cell-derived hepatocyte by two different 3D cell culture approaches. Both approaches were evaluated by measuring the expression level of key Cytochrome P450 (CYP) family members. 3D cell culture conditions were used with our existing ReproHepato cell product, and CYP expression was compared to that in conventional 2D cell monolayer. When 3D cultivation was applied to ReproHepato hepatocytes, basal CYP3A4 activity and CYP3A4 induction were increased to more than twice the level achieved with 2D cell cultivation. We also examined a 3D cell culture approach to the hepatocyte differentiation process. 3D cell culture of hepatocyte progenitor cells during the course of differentiation increased the basal CYP3A4 activity rise to nearly the same level as primary hepatocytes, and CYP induction was increased 5-fold. Furthermore, the basal expression levels of CYP1A2 and CYP2B6 also increased roughly 20-fold compared to using 2D cell culture. In conclusion, we have shown that 3D culture systems yield human IPS cell-derived hepatocytes of greater maturity. Furthermore we propose the use of IPS cell-derived hepatocyte panels as a way to cover all key CYP families and isotypes to compensate for inevitable donor variations. These approaches used in combination may be an attractive model for applications in drug discovery or small molecule evaluations.

Materials & Methods

ReproHepato type I™ kit (1 plate, 96-well) (ReproCELL #RCESDH001)
- Cells 1 vial (8.25 million cells/vial)
- Thawing Medium 1 bottle
- Maintenance medium 1 bottle
- Assay Medium 1 bottle
- Supplements

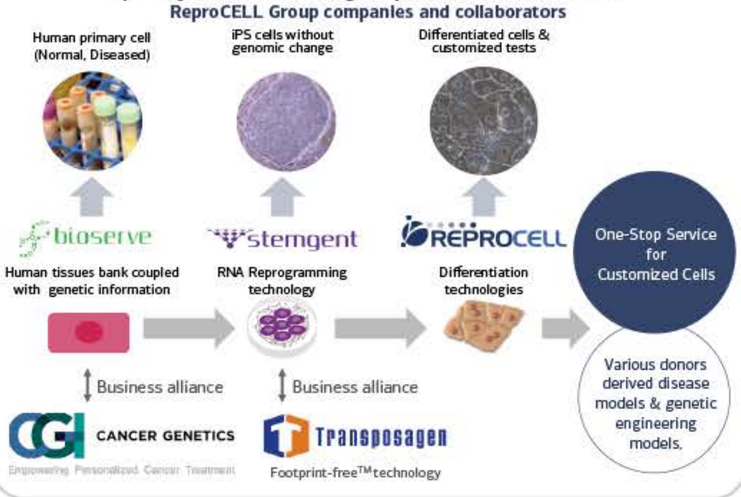
3D cell culture
- Puramatrix™ (3D Matrix Inc.)
- Nanoshuttle PL™ (n3D Bio Inc.)
- Low attachment plate (Sumitomo Bakelite)

RT-PCR
- CYP3A4 TaqMan® Gene Expression Assays (Life Technologies, Cat.No. Hs00604506_m1)
- CYP1A2 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs00167927_m1)
- CYP2B6 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs04183483_g1)
- CYP2C9 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs02383631_s1)
- CYP2C19 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs00426380_m1)
- CYP2E1 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs00559368_m1)
- CYP2A6 TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs00868409_m1)
- GAPDH TaqMan Gene Expression Assays (Life Technologies, Cat.No. Hs02758991gm1)

CYP3A4 induction assay
- Rifampicin (Sigma, Cat.No. R7382)
- Omeprazole (Sigma, Cat.No. 104)
- Sodium Butyrate NA (Sigma, Cat.No. 303410)
- P450-Glo™ CYP3A4 Assay with Luciferin-IPA (Promega, Cat. No. V9002)

Custom Human iPS Cell-Derived Hepatocytes

Specialty services combining cell products and services from ReproCELL Group companies and collaborators



ReproHepato CYP450 Expression in 2D Cell Culture

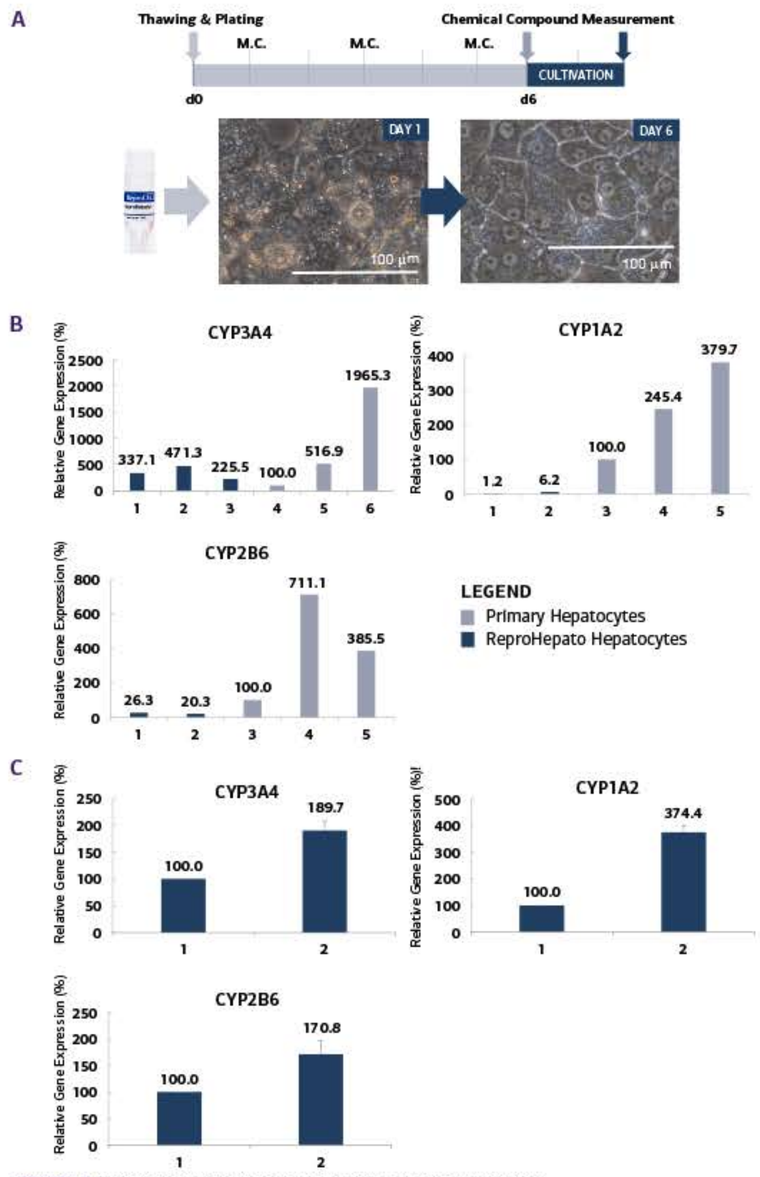


FIGURE 1: ReproHepato hepatocytes show CYP activity and induction
A. ReproHepato cells were thawed and seeded onto Corning® Matrigel®-coated 24-well plates. **B.** The basal expression levels of CYP3A4, 1A2, and 2D6 for bars 1,2,3 show different lots of ReproHepato iPS cells and bars 4, 5, 6 show different donors of Human Primary Hepatocytes. The CYP3A4 basal activities of primary hepatocyte lot 4 was taken as 100% and used to normalize the expression of other samples. **C.** The average induction levels of CYP3A4, 1A2, and 2B6 from 3 different ReproHepato lots. The average basal level of ReproHepato (bar 1) was taken as 100% and compared to the 10 µM Rifampicin induced level (bar 2). The error bar indicates standard deviation.

3D Cultivation of ReproHepato

FIGURE 2: 3D cultivation in Puramatrix improves the maturation of ReproHepato
A. Schematic for thawing, plating and assay of ReproHepato Type1 utilizing Puramatrix. **B.** The basal and induction level of CYP3A4 of ReproHepato in 2D (blue bar, 1) and on Puramatrix (blue bar, 2) is compared to different lots of primary hepatocytes (yellow bars, 3, 4 & 5) grown in 2D culture. The number on the graph above each bar shows relative expression after normalization to the basal level of primary hepatocyte lot 3.

3D Cultivation of ReproHepato

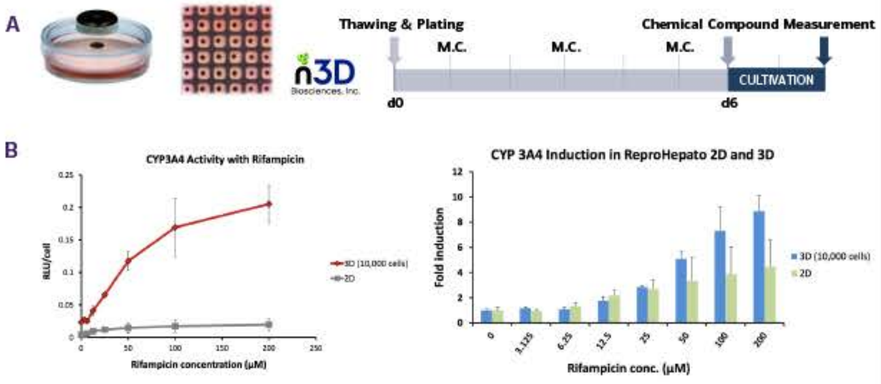
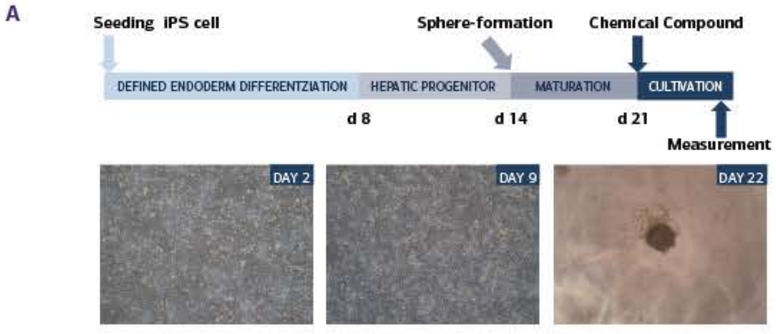


FIGURE 3: Human iPSC-hepatocyte spheroids demonstrate superior CYP3A4 activity and inducibility over 2D monolayer culture
A. ReproHepato hepatocytes were thawed and immediately plated creating a 2D monolayer (75,000 cells, 96-well format). ReproHepato 3D spheroids were prepared with magnetic 3D bioprinting (Nano3D Biosciences) by magnetizing the cells for 2 hours with Nanoshuttle-PL and printing them into spheroids with magnetic forces a(10,000 cells/spheroid, 384-well format). Hepatocytes were cultured for 7 days before exposure to rifampicin (0-200 µM). After a 72 hour exposure to rifampicin, CYP3A4 activity was measured using the P450-Glo assay (Promega). B. Induced CYP3A4 activity of ReproHepato cells by rifampicin was superior in spheroids than they were in monolayers.

Ongoing Research – 3D Culture Hepatic Differentiation



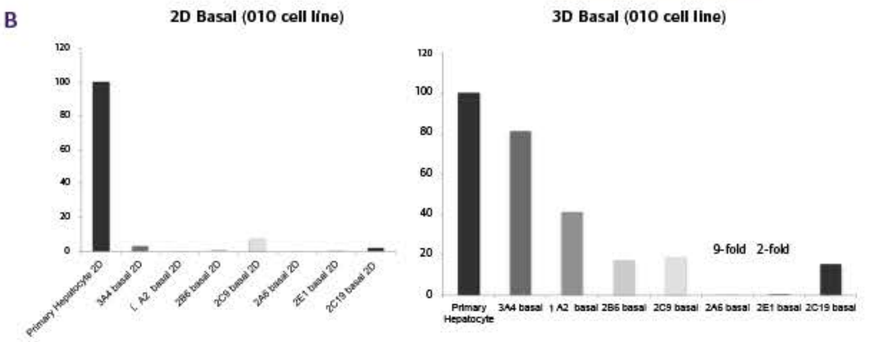


FIGURE 4: 3D cultivation during differentiation greatly improves the CYP expression of iPSC-derived hepatocytes
A. Schematic diagram of hepatic differentiation and the morphology of cells and colonies. **B.** Basal level of CYP expression after 2D-(left) and 3D-(right) cultivation and differentiation. In 2D-differentiated hepatocytes, the overall expression was low compare to primary hepatocyte. In 3D-differentiated hepatocytes, CYP3A4, 1A2 and 2B6 expression levels have greatly increased. CYP2C9 and 2C19 were also increased to a lesser extent, while expression of CYP2A6 and 2E1 showed increases in expression levels but still low basal levels. The basal level of CYP-expression of primary hepatocytes was taken as 1 and used to normalize the expression of all samples. Normalized values are shown.

Conclusion

- 3D cell culture systems are able to enhance the maturity of human IPS cell-derived hepatocytes as demonstrated by elevated CYP expression.
- Spheroid formation of hepato-progenitor cells prior to maturation has the greatest effect on basal level expression and inducibility of CYP enzymes.
- Basal level expression and inducibility of CYP in primary human hepatocytes is highly variable from lot to lot due to donor (genetic) variation.
- Unlike primary human hepatocytes, different lots of ReproHepato IPS cell-derived hepatocytes are highly consistent in basal expression levels of CYP enzymes.

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