**Introduction**

Human primary hepatocytes are utilized for high-throughput screening in early-stage drug discovery in order to evaluate thousands of potential compounds. Yet, human primary hepatocytes have the disadvantage of a limited supply from a single donor as well as high donor-to-donor variability. To overcome these obstacles, functional human induced pluripotent stem (iPS) cell-derived hepatocytes are highly desirable, as they are available in unlimited quantities from the same donor. However, immaturity and donor-to-donor variability are common drawbacks of iPS cell-derived hepatocytes. To address hepatocyte maturation, we evaluated multiple methods using 3D cultivation for maturing iPS cell-derived hepatocytes. We compared different 3D culture systems with traditional 2D cultures by analyzing the expression levels of specific cytochrome P450 (CYP) enzymes that play an important role in drug metabolism. We believed that 3D culture is able to provide a micro-environment that promotes maturation of human iPS cell-derived hepatocytes, potentially facilitating the creation of a human iPS cell-derived hepatocyte panel, which will enable assessment of donor-to-donor variability in iPS cell-derived hepatocyte function.

**Materials and Methods**

- **ReproHepato type™ kit** (Cat. No. RCESDH001)
  - Cells 1 vil (8.25 million cells/vial)
  - Thawing Medium 1 bottle
  - Maintenance medium 1 bottle
  - 24-well assay plate
  - Supplements

- **3D culture**
  - Puramatrix® (3D Matrix Inc.)
  - Nanoshuttle™ PL (N3O Bio Inc.)
  - Low attachment plate (sumitomo baleale)
  - Bioreactor (Alpha-Biot 30 ml)

- **RT-PCR**
  - CYP3A4 TaqMan Gene Expression Assay (Life technology, Cat.No. Hs00600450_m1)
  - CYP2A2 TaqMan Gene Expression Assay (life technology, Cat.No. Hs00165792_m1)
  - CYP2B6 TaqMan Gene Expression Assay (life technology, Cat.No. Hs00177043_m1)
  - CYP2C9 TaqMan Gene Expression Assay (Life technology, Cat.No. Hs00283361_x1)
  - CYP2C19 TaqMan Gene Expression Assay (life technology, Cat.No. Hs00426380_m1)
  - CYP2E1 TaqMan Gene Expression Assay (Life technology, Cat.No. Hs00559366_m1)
  - CYP2A6 TaqMan Gene Expression Assay (Life technology, Cat.No. Hs00864809_m1)
  - GAPDH TaqMan Gene Expression Assay (Life technology, Cat.No. Hs00279913_g1)

- **CYP3A4 induction assay**
  - Rifampicin (Sigma, Cat.No. R7382)
  - Omeprazole (Sigma, Cat.No. Ta1404)
  - Sodium Butyrate NA (Sigma, Cat.No. 303410)
  - P450-Glo™ CYP3A4 Assay with Luciferin-IPA (Promega, Cat. No. V9002)

**Characterization of ReproHepato**

3D cultivation of ReproHepato

** Optimization of Hepatic Differentiation: 2D Preliminary Study**

**Application of Preliminary Study to 3D Culture**

**Conclusion**

- Cultivation of ReproHepato Type 1 in 3D culture increases both basal level and induced CYP3A4 expression to a greater extent than primary hepatocytes.
- 3D spheroid-formation during hepatic differentiation improves the basal expression level of all 3 class expression further.