Novel culture medium that is able to maintain high CYP activity in human primary hepatocytes

Rina Akahira[1] , Zachary Yu-Ching Lin[1], Kenichi Tamura[1], Rina Akahira[1], Tomohisa Watanabe[1], Robert R. Annand[2], Roseann Vardaro[2], Toru Kiyono[3] and Mitsuru Inamura[1]

[1]ReproCELL Inc., KDX Shin-Yokohama 381 Bldg. 9F. 3-8-11, Shin-Yokohama, Kohoku-ku, Yokohama, Kanagawa 222-0033, Japan
[2]Stemgent Inc., 4 Hartwell Place, Lexington, MA 02421 USA
[3]National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

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Introduction

Hepatocytes play a critical role in metabolism of drugs which may otherwise have toxic effects in human liver. The use of cultured human primary hepatocytes to evaluate drug metabolism is widely adopted, and guidelines have been issued by the FDA and EMA regulatory organizations. However, primary hepatocytes rapidly lose their ability to express key metabolic enzymes from the cytochrome P450 (CYP) gene families. Typically, CYP activity declines to undetectable levels in about 96 hours. This instability causes logistical problems and higher costs for drug screening *in vitro* assays. In this study, we developed a new cell culture medium that maintains high CYP activities of human primary hepatocytes up to 96 hours in culture. Based on rational design we first screened (qPCR assay) many combinations of different physical parameters, chemical inhibitors and biological compounds (nutrients, growth factors, etc.) for the ability to increase CYP3A4 activity in culture. Finally, ten different promising medium compositions were further characterized by measuring CYP activity after 96 hours of cell cultivation.

One novel composition was selected from the candidates based on performance, stability, cost and other criteria. We then compared this novel culture medium with a popular brand of commercially available cell culture medium for human primary hepatocytes. Expression of CYP3A4 and other CYP enzymes were measured by using the qPCR assay. Hepatocytes cultured with our new medium show higher levels of expression for all CYP enzymes tested compared to the alternative brand medium. We further improvement expression levels by seeding human primary hepatocytes on top of the Alvetex scaffold (Reinnervate, Inc.), providing a 3D cultivation system to test with the new medium. As judged by qPCR, the basal and induced expression of CYP3A4 in 3D culture was elevated relative to monolayer culture. Furthermore, CYP activity of ReproHepato™ (iPS cell-derived hepatocytes), can also be improved when cultured in the medium. In conclusion, the new formulation shows high performance for *in vitro* drug metabolism studies with improved CYP expression, induction levels, and stability of expression over time in both 2D and 3D culture formats, while maintaining a highly distinct cobblestone morphology characteristic of mature human hepatocytes.

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 culture
- Alvetex® Scaffold (Reinnervate #AVP005)

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)

Hepatocyte Medium Overview

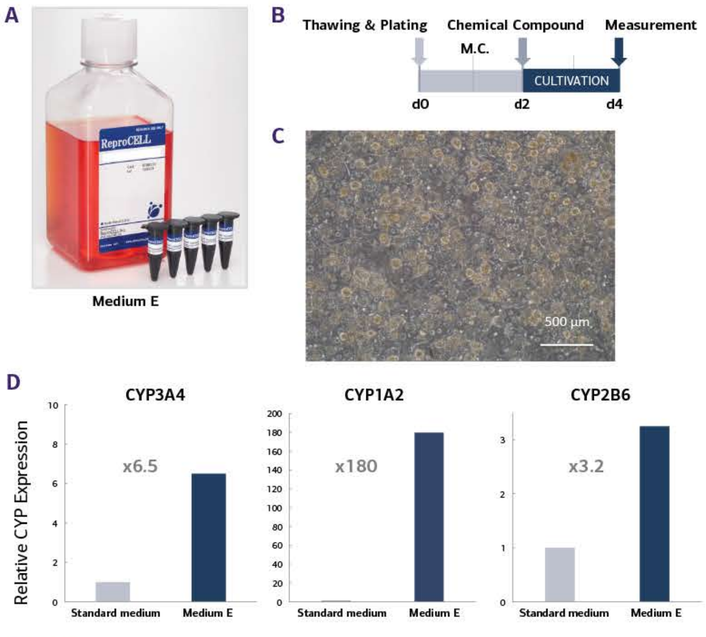

FIGURE 1: Summary of Medium E – ReproCELL High Performance Medium
A: Medium E consists of basal medium and supplements. **B:** Medium E can be used for culturing of primary hepatocytes and is compatible with conventional CYP assays. **C:** The morphology of primary hepatocytes cultured by using Medium E. **D:** As judged by qPCR, the basal level expression in primary hepatocytes of the 3 CYP enzymes tested (3A4, 1A2, 2B6) are elevated when cells are cultured in Medium E compared to a leading standard medium. CYP3A4 increased about 6.5-fold, CYP1A2 increased about 180-fold, and CYP2B6 increased about 3.2-fold for cells cultured in Medium E.

Cell Culture Media Development

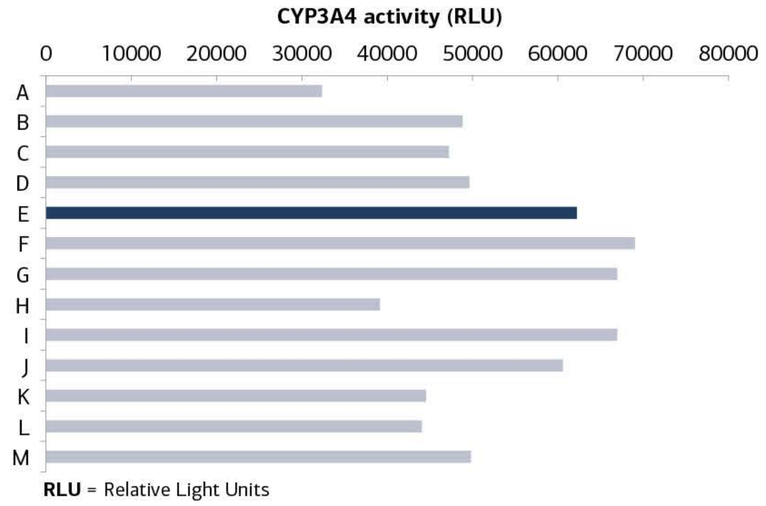

FIGURE 2: Screening for new hepatocyte high-performance culture medium development
In the development of Medium E, we screened various compositions of nutrients for the ability to increase CYP3A4 activity in primary hepatocyte culture. The data shown represents 13 different medium compositions evaluated on primary hepatocytes. After 96 hours of culture, CYP3A4 activities were assayed by using P450-Glo™ CYP3A4 kit with Luciferin-IPA (Promega). We found several nutrient compositions that gave elevated CYP3A4 activity in cultured primary hepatocytes. Medium E was selected for further study based on stability, cost and other considerations.

CYP Expression Time Course in Medium E

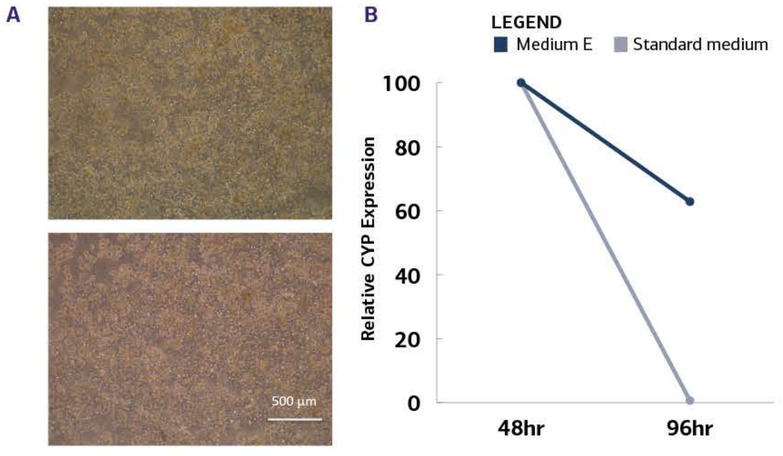

FIGURE 3: High primary hepatocyte CYP expression is maintained during culture in Medium E
A: The morphology of primary hepatocytes cultivated in Medium E (top) and competitor standard medium (bottom) are similar. **B:** The analysis of CYP3A4 activity between 48 hours to 96 hours after thawing shows that Medium E maintained primary hepatocytes expression longer than standard medium. The CYP expression of primary hepatocytes cultivated in competitor standard medium decreased to 1% within 96 hours. The activity of primary hepatocytes at 48 hr was taken as 100%.

CYP Expression Induction in Medium E

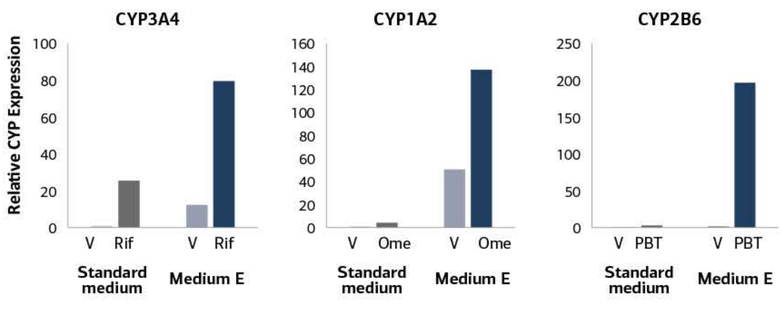

FIGURE 4: CYP induction is dramatically increased with Medium E
CYP3A4, 1A2 and 2B6 induction assay in primary hepatocytes cultivated in both competitor standard medium and Medium E. After 48 hours of culture with Medium E or standard medium, each inducer was applied and incubated further for 48 hours. CYP expression was measured by using qPCR.
V: Vehicle, Rif: 10 µM rifampicin, Ome: 50 µM omeprazole, PBT:1 mM phenobarbital
The graph shows relative expression . The basal level of primary hepatocytes was taken as 1.

3D Cultivation with Medium E

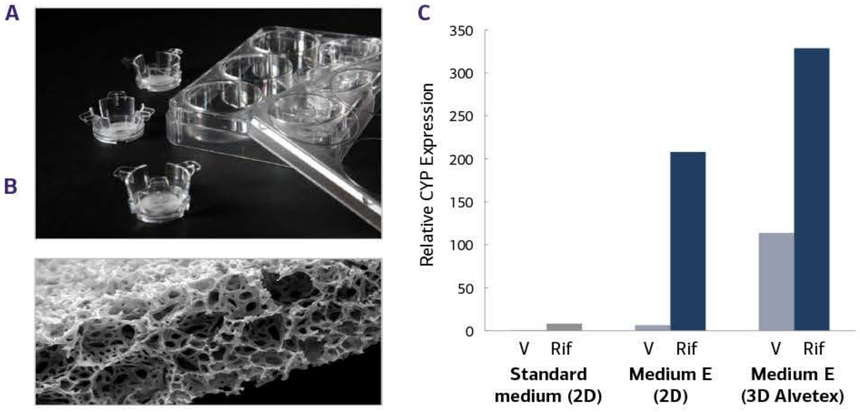

FIGURE 5: Medium E combined with 3D culture improve CYP3A4 expression in primary human hepatocytes.
A,B: Alvetex (Reinnervate, Inc.) 3D culture Support (A; hanging insert format, B; high magnification). **C:** qPCR assay of CYP3A4 induction-shows that basal expression and induction levels in primary hepatocytes grown in 3D culture with Medium E (right) were increased to the greatest extent. The 3D plus Medium E combination increased CYP3A4 expression greater than standard primary hepatocyte medium (Medium A; left) or Medium E alone (middle). V: Vehicle only, Rif: 10 µM rifampicin.

ReproHepato Cultured in Medium E

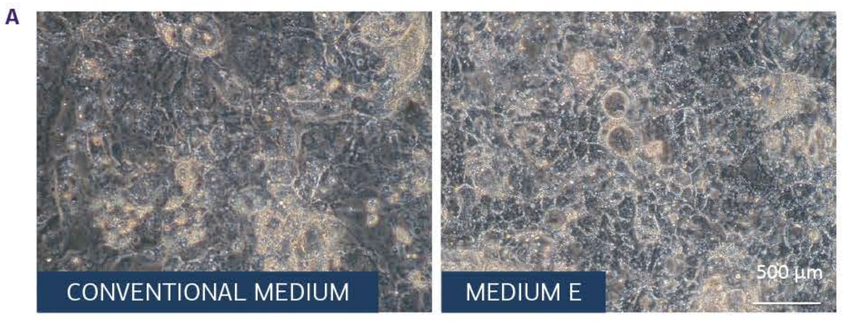

FIGURE 6: CYP3A4 expression of ReproHepato (human iPS cell-derived) hepatocytes is improved when cultured with Medium E
A: The morphology of ReproHepato in different conditions (left: conventional hepatic differentiation medium. Right: Medium E). **B:** qPCR analysis shows that growth in Medium E improves CYP3A4 basal and induced expression in ReproHepato cells.

Conclusion

- Higher CYP enzyme induction levels and overall activity in human primary hepatocytes can be achieved by growth in the new high-performance hepatocyte medium, Medium E.
- High levels of CYP enzyme activity remain up to 96 hours in cultured human primary hepatocytes grown in Medium E.
- Medium E and 3D culture when combined improve CYP3A4 activity versus 2D monolayer culture.
- The activity of CYP3A4 in ReproHepato (human iPS cell-derived hepatocytes), is also improved by cultivation in Medium E.

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