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Characterization of a human hepatocellular carcinoma cell line on micro-space cell culture plate.

○Yoko Ejiri[1], Akane Yoshida[2], Masaya Hosoda[1], Kaoru Kobayashi[2] and Kan Chiba[2]
(1)Tsukuba Research Center, Kuraray Co., Ltd., Tsukuba, Japan, (2)Laboratory of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Contact information:
Yoko Ejiri
E-mail: youko_ejiri@kuraray.co.jp

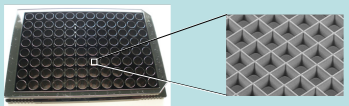
Abstract

We evaluated a novel three dimensional culture plate, Micro-space cell culture plate, with human hepatocellular carcinoma cell line, FLC-4. The plate has micrometer sized compartments regularly arrayed on its surface. To optimize cell culture conditions, FLC-4 cells were cultured on two micro-patterns (200μm width X 100μm depth and 200μm width X 50μm depth). Also, surface coating and seeding density were optimized. As a result, FLC-4 formed spheroid on the 200μm X 100μm pattern having hydrophilic surface at day 7. mRNA expression of CYP1A1, 1A2, 2C9, 3A4 and UGT on 200μm X 100μm micro-space plate were higher than those in monolayer culture. mRNA expression of CYP3A4 and UGT1A1 were significantly induced by phenobarbital on 200μm X 100μm pattern plate.

Purpose

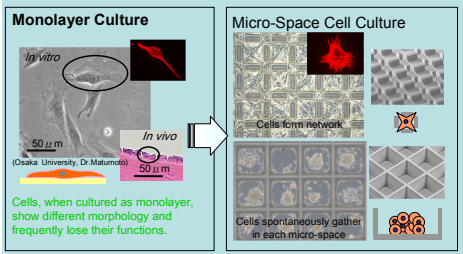
1. Optimization
   - The pattern of the Micro-space cell culture plate
   - Surface coating
   - Cell density
   - Culture periods
2. Induction test

Micro-space cell culture plate



The bottom of micro-space cell culture plate consists of spaces range from several to several hundred micrometers. When cultured in the space, certain types of cell form 3-D structure which mimics *in vivo* morphology and promotes better functions.

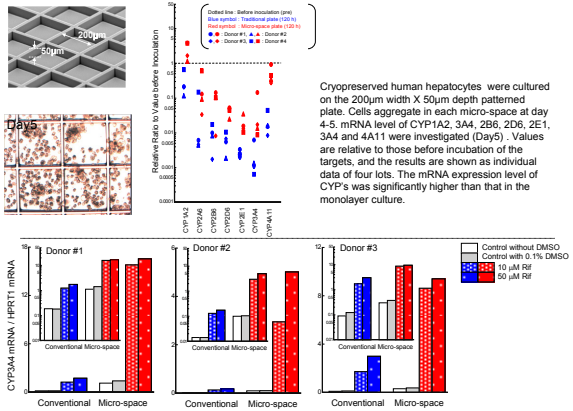
About "Micro-space cell culture plate"



By providing micro spaces on the surface of cell culture plate, certain types of cells form 3-D structure which mimics in vivo morphology and promotes better functions.

Cell Culture Examples

Cryopreserved human hepatocytes

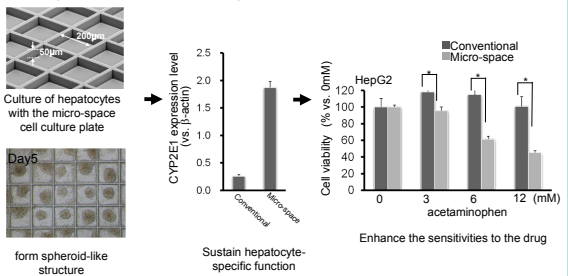


Cryopreserved human hepatocytes were cultured on the 200μm width X 50μm depth patterned plate. Cells aggregate in each micro-space at day 4-5. mRNA level of CYP1A2, 3A4, 2B6, 2D6, 2E1, 3A4 and 4A11 were investigated (Day5) . Values are relative to those before incubation of the targets, and the results are shown as individual data of four lots. The mRNA expression level of CYP's was significantly higher than that in the monolayer culture.

The changes in CYP3A4 mRNA level induced rifampicin (Rif) in cryopreserved human hepatocytes from 3 donors using conventional and micro-space cell culture plates are shown in Fig. On conventional plate, the level of CYP3A4 mRNA increased by 9.4-, 12- and 24-fold, respectively, after exposure to 50 μM Rif, compared with that in 0.1% DMSO-treated controls, while on micro-patternedspace plate cultures, the level increased by 12-, 48- and 25-fold, respectively.

Drug Metab Pharmacokinet. 2011;26(2):137-44.
Drug Metab Pharmacokinet. 2010;25(3):236-42.

Human hepatocellular carcinoma HepG2 cells

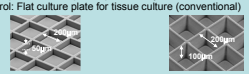


HepG2 cells were cultured on the 200μm width X 50μm depth patterned plate. Cells formed spheroid-like structure in each micro-space. The CYP2E1 mRNA expression level of HepG2 in the micro-space cell culture was significantly higher than that in the monolayer culture. The cells in the micro-space cell culture were more sensitive to acetaminophen-induced hepatotoxicity than those in the monolayer culture.

J Biosci Bioeng. 2011 Jan;111(1):78-84

Materials and methods

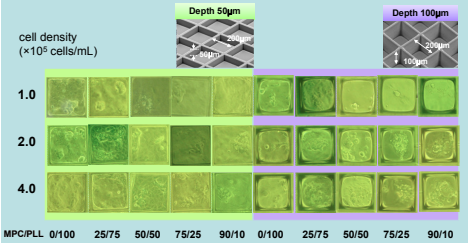
➢Culture plate
- Two types of the Micro-space cell culture plates (Micro-space) were used. These plates have square spaces arrayed on the bottom of 24-well plates.
- Control: Flat culture plate for tissue culture (conventional)



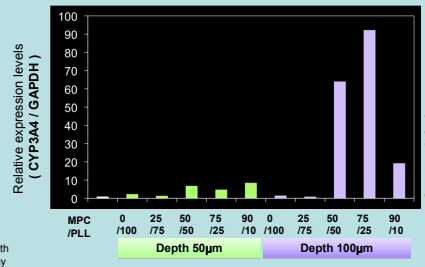
D7-200-50 (Depth 50μm) D7-200-100 (Depth 100μm)

➢Cell
Human hepatocellular carcinoma cell line FLC-4 cell
➢Medium
10% heat-inactivated fetal bovine serum , 100 units/mL penicillin G and 100 μg/mL streptomycin in Dulbecco's modified Eagle's/Ham's F12 medium
➢Coating
The micro-space cell culture plates were coated with a mixture of PLL*1 and MPC*2 solution. Conventional plate were not coated.
*1: 0.01% poly-L-lysine solution, MW: 150,000-300,000 (PLL)
*2: 0.01% 2-methacryloyloxyethyl phosphorylcholine polymer solution (MPC)

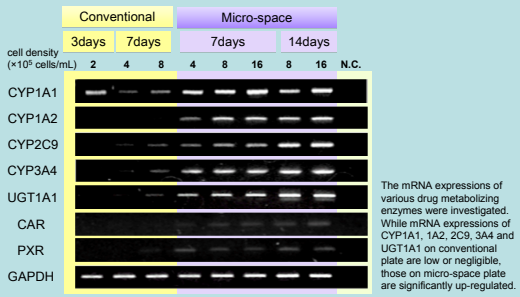
Result 1. Optimization



FLC-4 cells on micro-space cell culture plates began conglomerating and showed spheroid structure on Depth 100μm. Regarding coating method, MPC/PLL ratio; 50/50, 75/25, 90/10 provided spheroid architecture at day 7 in each compartment. Larger spheroids were observed in higher cell density. The cell morphology by other condition was similar to conventional.



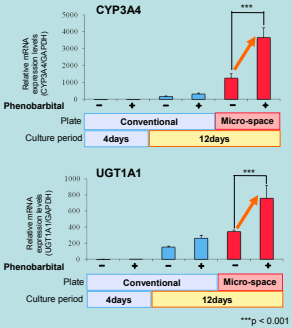
The CYP3A4 mRNA expression correlates to the cell morphology. Cells formed spheroid on Depth 100μm with surface coating MPC/PLL: 50/50 and 75/25, and mRNA expression was enhanced on those conditions.



The mRNA expressions of various drug metabolizing enzymes were investigated. While mRNA expressions of CYP1A1, 1A2, 2C9, 3A4 and UGT1A1 on conventional plate are low or negligible, those on micro-space plate are significantly up-regulated.

Culture period and cell density were investigated in more detail for Depth 100um and MPC/PLL: 90/10 in which conditions spheroids formed. Larger spheroids were observed in higher density and longer (14 days) culture period.

Result 2. Induction test



***p < 0.001

The inducibility of CYP3A4 and UGT1A1 mRNA on micro-space plate by exposure to phenobarbital is similar to that on conventional, however, the amount of the mRNA of these enzymes expressed on micro-space plate was higher than that of conventional.

Conclusion

Human hepatocellular carcinoma cell line, FLC-4, cultured on micro-space cell culture plate, showed markedly higher mRNA expression of drug metabolizing enzymes than that on conventional plate. To enhance the metabolism of FLC-4 on micro-space plate, culture conditions including cell density, culture period and surface coating should be optimized. Micro-space cell culture is suggested to be a useful tool for drug metabolism study with cell lines such as enzyme induction and cytotoxicity studies mediated by drug metabolizing enzymes.