Bioscience Solutions

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Comparison of Hepatocytes in Monolayer and RAFT™ 3D Cell Culture System

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Introduction

Hepatocytes are the primary cell type of the liver and function to provide the majority of the detoxification in the body. Typical *in vitro* drug toxicity screening has incorporated hepatocytes grown in a two dimension (2D) format, cultured between collagen coated tissue culture plastic and Matrigel ("Sandwich Model"). The potential of three dimension (3D) models to better mimic the *in vivo* cellular environment have made them attractive alternatives to 2D culture systems.

Lonza offers a novel RAFT™ (Real Architecture For Tissue) 3D Culture system which allows the creation of tissue-like structures. The 3D matrix of the type 1 collagen-based RAFT™ Culture provides a more natural cell culture environment and therefore a potentially superior model for in vitro screening.

Here we compare cell viability and cell morphology of rat and human hepatocytes, and the maintenance of Cytochrome P450 (CYP) activity in human hepatocytes grown in the traditional Sandwich Model with that of cells cultured in the 3D RAFT™ System. Our results show that the RAFT™ 3D System represents a more robust model for the long-term maintenance of liver-specific functions.

Materials and Methods

Cell Culture

Freshly isolated primary rat (Sprague-Dawley) hepatocytes and cryopreserved human hepatocytes were used for the study. All cultures were fed daily.

Sandwich Model: Hepatocytes were plated on collagen coated 96-well plates (Corning, Cat No. 354407) in complete Hepatocyte Plating Medium (Lonza, Cat No. MP100-1) or incomplete Hepatocyte Culture Medium (HCM, Lonza, Cat No. CC-3199 and CC-4182) at the cell densities indicated. Cells were overlayed with 0.3 mg/mL Matrigel (Corning, Cat No. 354234).

RAFT™ 3D Culture System Model: RAFT™ Kits (Cat No. 016-0R94) and absorbers (Cat No. 016-0R92) were obtained from Lonza. Cells were embedded in one quarter of the recommended volume of collagen matrix (60µL, Figure 1) at the cell densities indicated on 96 well tissue culture plates.

Assay Kits

The following kits were used as per manufacturers' instructions: The CellTiter-Glo® 3D Viability Assay (CTG, Promega, Cat No. G9681), alamarBlue™ Cell Viability Reagent (Thermo, Cat No. DAL 1025), P450-Glo™ CYP 1A2, 2B6, and 3A4 Assays (Promega, Cat No. V8421, V8321, V9001).

Immunofluorescence

Cultures were fixed with ice cold Methanol and blocked with 10% Normal Goat Serum. Cells were stained for tight junctions with ZO-1 antibody (Thermo, Cat No. 33-9100). The secondary antibody used was Alexafluor 555 (Thermo, Cat No. A21425). Appropriate isotype controls were incorporated.

CYP450 Activity Quantitation

Cells were induced with Omeprazole (1A2), Phenobarbital (2B6) or Rifampicin (3A4) for 72 hours. The corresponding substrates Phenacetin, Bupropion or Testosterone were applied and the culture supernatants were collected. Basal CYP activities of non-induced cells were also evaluated. Metabolite quantification was determined by Liquid Chromatography Mass Spec (LCMS).

Figure 1. RAFT™ 3D Cell Culture Process Overview

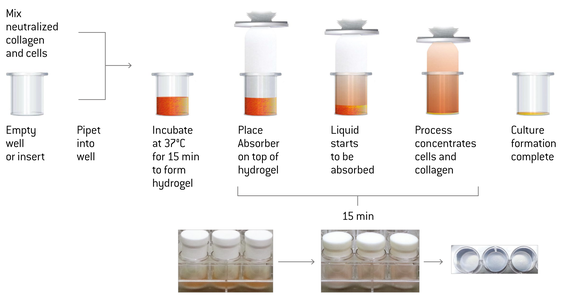

Figure 2. Fresh Rat Hepatocytes Survive for Extended Time in RAFT™ Cell Culture

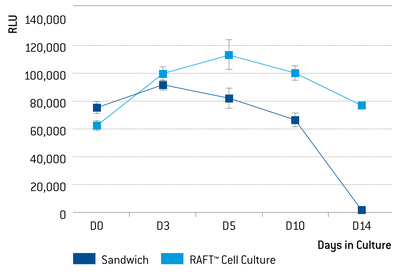

Figure 2. The viability of fresh rat hepatocytes cultured in the RAFT™ 3D Cell Culture and Sandwich Models were compared using the CellTiter-Glo® 3D Viability Assay. Cells were plated at 45,000 cells per well in each respective model. The long-term survival of rat hepatocytes is more robust in the RAFT™ 3D Culture System than in traditional Sandwich Culture.

Figure 3. Fresh Rat Hepatocytes Form Tight Junctions in RAFT™ Cell Culture and in Sandwich Culture

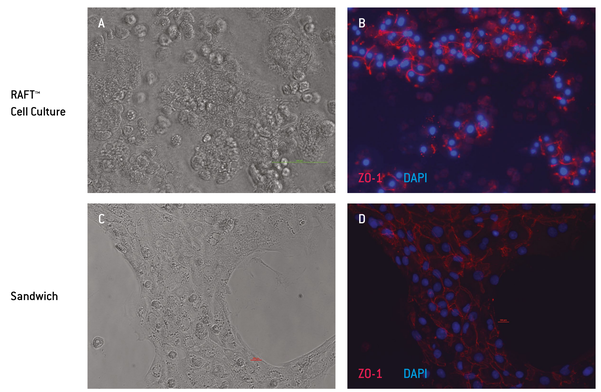

Figure 3. Fresh rat hepatocytes were plated at 45,000 cells per well in RAFT™ Cell Culture and Sandwich Models and cultured for 10 days. Methanol fixed cultures were stained for tight junctions with the ZO-1 antibody (red, **(B)** and **(D)**) and counter stained with DAPI (Blue, **(B)** and **(D)**). **(A)** Bright Field 20x RAFT™ Cell Culture **(B)** ZO-1 stained 20x RAFT™ Cell Culture **(C)** Bright Field 20x Sandwich **(D)** ZO-1 stained 20x Sandwich

Figure 4. Cryopreserved Human Hepatocytes Exhibit Higher Viability in RAFT™ Cell Culture than in Sandwich Culture

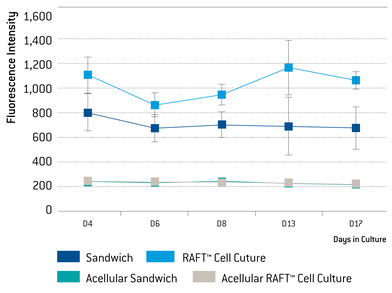

Figure 4. The viability of cryopreserved human hepatocytes cultured in the RAFT™ Cell Culture and Sandwich Models were compared using the alamarBlue™ Cell viability Reagent. Cells were plated at 65,000 cells per well in each respective model. The survival of human hepatocytes is more robust in the RAFT™ 3D Culture System than in traditional Sandwich Culture.

Figure 5. Human Hepatocytes Form Tight Junctions in RAFT™ Cell Culture

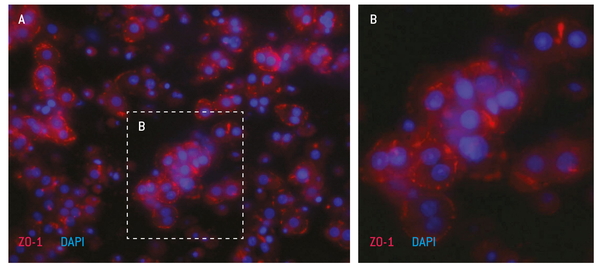

Figure 5. Cryopreserved human hepatocytes were plated at 65,000 cells per well in the RAFT™ System and cultured for 4 days. Methanol fixed cultures were stained for tight junctions with the ZO-1 antibody (red) and counter stained with DAPI (blue). **(A)** ZO-1 stained RAFT™ Cell Culture 20x. Inset is enlarged **(B)** to show detail.

Table 1. CYP Inducers, Substrates and Metabolites

CYP450 Isoform	Inducer	Substrate	Metabolite
CYP1A2	50 µM Omeprazole	100 µM Phenacetin	Acetaminophen
CYP2B6	1 mM Phenobarbital	250 µM Bupropion	0H-Bupropion
CYP3A4	10 µM Rifampicin	200 µM Testosterone	6ß-Hydroxytestosterone

Table 1. Human Hepatocytes were plated at 65,000 cells per well in RAFT™ Cell Culture and Sandwich Models and incubated with appropriate inducers for 72 hours. Supernatants from induced and uninduced cells were analyzed using the P450-Glo™ Assays **(Figure 6A, 7A, 8A)**. In separate experiments the induced and uninduced cells were incubated with the appropriate substrate and LCMS was performed to quantitate metabolites **(Figure 6B, 7B, 8B)**.

Table 2. Fold Induction of CYP activity on day 16/17

	P450-Glo™ Assay		LCMS	
	Sandwich	RAFT™ Cell Culture	Sandwich	RAFT™ Cell Culture
CYP1A2	4.8	6.4*	29.6	6.0*
CYP2B6	1.2	0.3*	4.5	12.4*
CYP3A4	15.3	61.8*	4.2	33.6

Table 2. Fold induction was calculated from the induction and basal CYP activity for both direct and indirect methods shown in Figure 6. *Basal CYP levels higher in RAFT™ Cell Culture (Figure 6).

Summary

- Rat and human hepatocytes survive and remain more metabolically active for longer periods of time in the RAFT™ 3D Culture System than in the 2D Sandwich Model.
- Rat and human hepatocytes maintain the ability to form tight junctions in both RAFT™ Cell Culture and Sandwich Culture.
- CYP450 activity of human hepatocytes is higher for longer culture durations in RAFT™ 3D Culture System than in Sandwich culture.

Conclusion

Hepatocyte metabolism is stabilized in the RAFT™ 3D Culture System which better enables long-term toxicity analysis using primary hepatocytes. The RAFT™ 3D Culture System is easy to use, providing a robust model for assessing liver toxicity.

Figure 6. The Basal and Induced CYP Activities in Human Hepatocytes are Higher in RAFT™ Cell Culture than in Sandwich Cultures

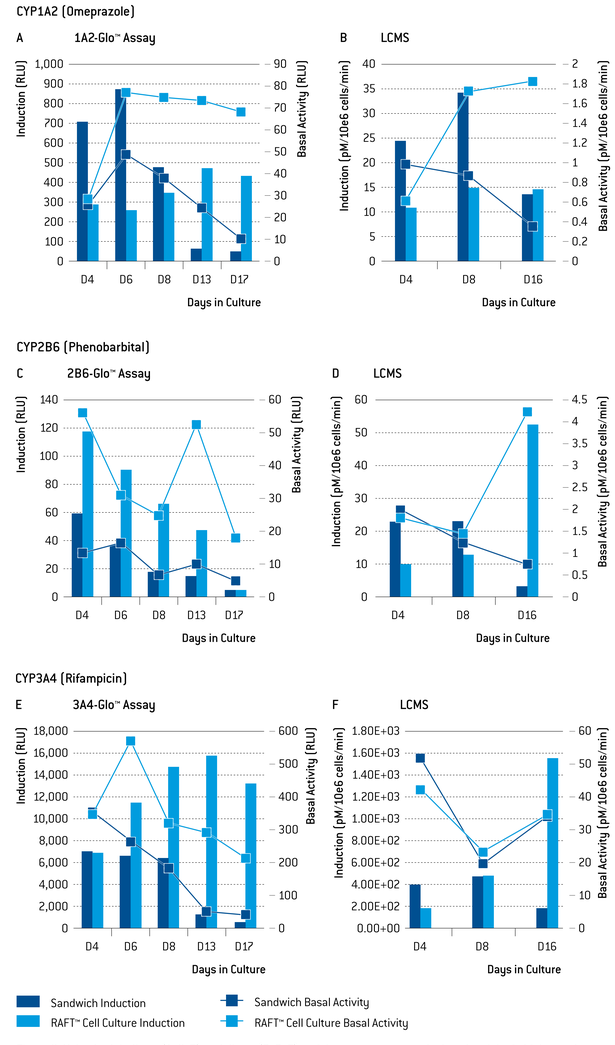

Figure 6. Using both indirect (A, C, E) and direct (B, D, F) activity measurements, the basal activity is higher and more stable when cultured in the RAFT™ System. Omeprazole (CYP1A2) induction levels, however, were higher in Sandwich cultures up to day 8 of culture. Reduced induction levels observed in RAFT™ Cell Culture in later culture durations could be result of higher basal activity.