Using the RAFT™ System as a versatile tool to build in vitro models relevant for toxicity testing

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Introduction

Conventional in vitro assays are based on cells grown on two-dimensional (2D) substrates, which are not representative for the true in vivo cell environment. In tissue, cells interact with neighboring cells and with the extracellular matrix [1]. Three-dimensional (3D) cell culture methods mimic these interactions and allow cells to grow in structures resembling more the in vivo environment.

The RAFT™ 3D Culture System uses a collagen matrix at physiologically relevant concentrations. The resulting models enable long-term drug metabolism studies and toxicity analysis. • Primary hepatocytes survived and remained more metabolically active for up to 17 days in culture, which enables long-term drug metabolism studies and toxicology analysis.

The RAFT™ 3D Cell Culture System allows the co-culture of different cell types in physiological orientation. • In contrast to standard 2D cultures, undesired overgrowth of certain cell types is observed to a lesser extent in RAFT™ 3D cultures. • Phenotypic differences, for example in cell morphology or protein secretion, are detected when comparing cells cultured in the RAFT™ System to those in standard 2D culture.

Conclusion

• The RAFT™ 3D Cell Culture System provides a valuable tool to investigate different cell types in single or co-culture in an in vivo-like collagen-based microenvironment.

Materials and methods

Cell Culture

All cells were obtained from Lonza and cultured according to manufacturer’s protocols before being transferred into RAFT™ Cultures. The following cell types and culture media were used: Skin: Clonetics™ Human Dermal Fibroblasts (HDF™) with Fibroblast Growth Medium (FGM™–2) medium, Human Epidermal Keratinocytes (HEK) with Keratinocyte Basal Medium (KBM™–Gold) and KGM™ SingleQuots™ (KGM™–Gold SD). Lung Normal and Diseased (Asthma) Human Bronchial Epithelial (HBE, DBHE) with Bronchial Epithelial Cell Growth Medium (BECM™), Normal and Diseased (Asthma) Human Bronchial Smooth Muscle (BSMC, BSMD™) with Smooth muscle Growth Medium (SmGM™–2). Liver: For control cultures, 65,000 hepatocytes were plated on collagen coated 96-well plates [Corning, Cat. No. 354405] in complete Hepatocyte Plating Medium (Lonza, Cat. No. MP-100-1) or in complete Hepatocyte Culture Medium [KGM, Lonza, Cat. No. CC-3399 and CC-4182]. Cells were overlaid with 0.3 mg/mL Matrigel™ (Corning, Cat. No. 354234).

RAFT™ 3D Cell Culture System

RAFT™ 3D Cell Culture Kits were used for the formation of cell seeded collagen gels as per the manufacturer’s protocol. Briefly, cells and neutralized collagen were mixed and dispensed into wells of 96-well, 24-well, or 24-well insert cell culture plates, and subsequently incubated at 37°C to allow hydrogel formation. RAFT™ Absorbers were placed on top of the hydrogels. The absorbers gently remove abundant medium and compact the hydrogel to a layer approximately 100 μm thick (Figure 1). The cultures are then ready to use, but additional epithelial or endothelial cells may be added on top. For details about the individual models, please see individual tissue models sections.

Immunohistochemistry

Skin equivalent tissues were fixed in 45% paraformaldehyde and processed for histology. Sections were either Hematoxylin and Eosin stained or deparaffinized and labeled with the indicated antibodies. Other RAFT™ Cultures were fixed with methanol, washed with PBS and stained with the indicated antibodies (red) as well as DAPI (blue, nuclei).

ELISA

Spent media were collected for ELISA and analyzed using Raybiotech’s Quantibody® Human Angiogenesis Assay Array 3 testing service.

Liver model

A 3D liver model was created by embedding 65,000 cryopreserved primary human hepatocytes within the RAFT™ Collagen. Hepatocytes survived and remained more metabolically active for longer periods of time than in the traditional Sandwich Culture.

B 3D co-culture model containing normal or asthmatic bronchial epithelial and smooth muscle cells was created by embedding BSMC, followed by an overlay with HBE. Figure 2: Bronchial Epithelial/RAFT™ Model.

Figure 3. Creation of a RAFT™ 3D skin model

Figure 4. Histology of RAFT™ Skin model

Figure 5. Analysis of RAFT™ Liver models for up to 17 days of culture. Hepatocytes remain viable in RAFT™ Cultures for up to 17 days (A: Atrazine/Hepatocyte Cell Viability, B: Acetaminophen/Cell Viability, C: Atrazine/Hepatocyte Cell Viability). Images were taken with a 20x objective. (D) Insert enlarged to show detail. (F) Activity of the key drug metabolizing liver enzyme CYP2A6 was determined after 3 days of culture using the fluorescent CYP2A6 3A4 Assay as an indicator for measurement of the conversion of 3A4 substrate 7-ethoxycoumarin. Images were taken with a 20x Microscopy objective. (H) Insert to show detailed staining. (J) Activity of the key drug metabolizing liver enzyme CYP2A6 was determined after 3 days of culture using the fluorescent CYP2A6 3A4 Assay as an indicator for measurement of the conversion of 3A4 substrate 7-ethoxycoumarin. Images were taken with a 20x Microscopy objective.

Figure 6. RAFT™ Skin model

Figure 7. RAFT™ 3D culture process overview

Summary

• The RAFT™ 3D Cell Culture System allows the co-culture of different cell types in physiological orientation.

• In contrast to standard 2D cultures, undesired overgrowth of certain cell types is observed to a lesser extent in RAFT™ 3D cultures.

• Phenotypic differences, for example in cell morphology or protein secretion, are detected when comparing cells cultured in the RAFT™ System to those in standard 2D culture.

Skin model

A full-thickness skin model was generated by embedding NIH/3T3 Collagen in a 24-well transwell insert followed by a compression step according to RAFT™ Kit instructions. Subsequently, 65,000 of NIH/3T3 collagen solution was added without further compression. The dermis layer was allowed to develop for 5 days. After 48 hours, medium was exchanged to Cont.P/F-TSL® (CellTreat) and after 24 hours, cultures were air-lifted allowing keratinocyte differentiation for 10-12 days. The resulting cultures resembled actual human skin (Figure 4).

Conclusion

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