Cell culture and cell analysis using the Real Architecture for 3D Tissue (RAFT™) Culture System

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Introduction

Conventional in vitro assays are based on cells grown on two-dimensional (2D) substrates, which are not representative of a true in vivo cell environment. Three-dimensional (3D) cell culture methods, in contrast, allow cells to grow in structures more resembling the in vivo environment. Cells can develop cell-cell and cell-extracellular matrix (ECM) interactions in 3D.

While 3D cultures more accurately resemble the in vivo environment, it might be difficult to analyze cells in 3D. However, this is not always the case. This poster explains how standard analysis techniques, like fluorescence microscopy, can be applied easily to RAFT™ 3D Cultures. In addition, it will be shown how the RAFT™ Technology can be combined with transient transfection approaches using the Lonza Nucleofector® System.

Materials and Methods

For detailed protocols of the assays presented please visit www.lonza.com/raft.

RAFT™ 3D Culture System

The RAFT™ 3D Culture System uses a 220 µm thick collagen matrix at physiologically relevant concentrations. Cells like fibroblasts or smooth muscle cells can directly be embedded in the collagen matrix. Additional epithelial or endothelial cells may be added as overlays on top to study co-cultures or more complex cultures.

Figure 1. The RAFT™ Process

Fig. 1. A) Build and insert cell-laden hydrogel. B) 15 min

Figure 2. Cell Growth in and on RAFT™ 3D Cultures

Fig. 2. A) HCT 116 Cells were cultured for 6 days in RAFT™ 3D Cultures before being stained with Calcein AM for viable cells (green) and Propidium Iodide for dead cells (red). The image shows the stereo micrograph of this culture. B) Floating RAFT™ Cultures. Cells were cultured for 2 hours before being analyzed. Scale bar: 100 µm

Figure 3. Efficient Transfection for RAFT™ 3D Cultures

Fig. 3. A) The 4D-Nucleofector® System with its accessories – the 220 µL NucleoCuvette® (step 1), the 100 µL NucleoCuvette® (step 2), the 20 µL NucleoCuvette® (step 4) and the Absorber (step 3) – are ideal for use with RAFT™ 3D Cultures. Cells were seeded on top of RAFT™ 3D Cultures and incubated for 24 hours. Then, the cells were transfected with the ViaLight™ Plus BioAssay. Scale bar: 100 µm

Figure 4. Immunochemistry of RAFT™ 3D Cultures

Fig. 4. A) Immunochemistry Protocol for RAFT™ 3D Cultures. The concentration of Triton X-100 (Sigma-Aldrich) in the permeabilization and 1° antibody step is dependent on cell type. For cell lines that are sensitive to Triton X-100, less TX-100 is usually sufficient. For cell types like HCT 116 that form aggregates like structures in RAFT™ higher than 1% TX-100 concentration of 1%TX-100 is recommended. B) NIFD were cultured in the RAFT™ System for 24 h. Cells were stained for tubulin (red) and the nucleus (blue). One plane location was imaged on a widefield microscope. The image shows one channel. The merge of the two channels is shown below. Scale bar: 20 µm

Figure 5. ViaLight™ Plus Cell Proliferation and Cytotoxicity Bioassay

Fig. 5. A) Principle of the ViaLight™ Plus Bioassay. The emitted light can be detected using suitable luminometers or scintillation counters. B) Overview of the ViaLight™ Plus Bioassay Procedure. For RAFT™ 3D Cultures it is recommended to increase the lysis time (Step 3) from 10 minutes to 30 minutes to ensure efficient lysis of the approximately 100–120 µm thick cultures.

Figure 6. Performance of the ViaLight™ Plus Assay in RAFT™ 3D Cultures

Fig. 6. A) Relative ViaLight™ Luminescence Intensity (RLU) – vs. Time

References


Conclusions

— The RAFT™ 3D Culture System supports growth of various cell types in and on top of a physiologically relevant collagen scaffold.

— The RAFT™ 3D Culture System is compatible with transient transfection using the Lonza Nucleofector Technology.

— The RAFT™ 3D Culture System can be analyzed using standard cell biology methods like immunochemistry or the Lonza ViaLight™ Plus Bioassay.