BioResearch

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

Cecile Villemant[1], Sabine Schäpermeier[2], Stefanie Büsch[2], John Langer[3], Theresa D'Souza[3], Lubna Hussain[3], Grant Cameron[1], Volker Vogel[2], Jenny Schroeder[2]
[1]TAP Biosystems, Royston, UK; [2]Lonza Cologne GmbH, Cologne, Germany; [3]Lonza Walkersville Inc., Walkersville, MD, USA

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 Cell Cultures. In addition, it will be shown how the RAFT™ Technology can be combined with transient transfection approaches using the Lonza 4D 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 120 µm thick collagen matrix at physiologically relevant concentrations. Cells like fibroblasts or smooth muscle cells can be directly 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

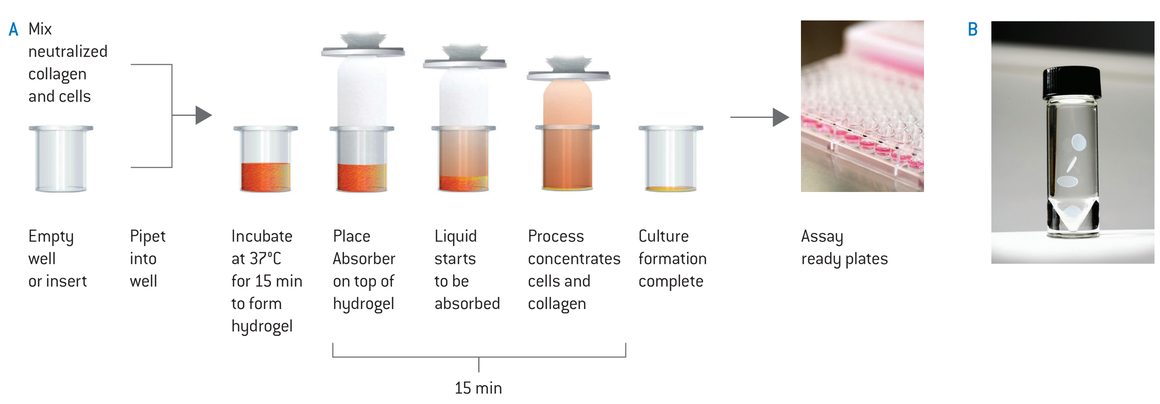

Fig. 1: A) The RAFT™ Process. Cell containing RAFT™ Cultures are formed within less than one hour. **B)** Floating RAFT™ Cultures.

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

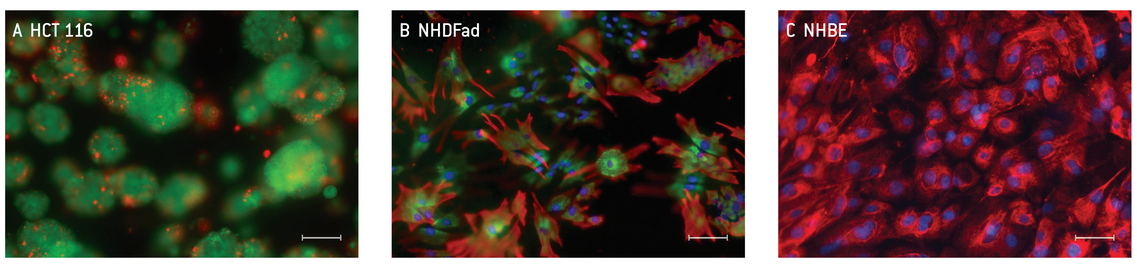

Fig. 2: A) HCT 116 cells were cultured for 6 days in RAFT™ 3D Cultures before being stained with Calcein AM for viable cells (green) and Propidium Iodide to detect dead cells (red). The colon cancer cell line formed tumor-like structures in RAFT™ 3D Cultures. **B)** Adult normal dermal human fibroblasts (NHDFad, Lonza Cat. No. CC-2511) were cultured for 7 days in RAFT™ 3D Cultures before being stained for type III intermediate filament Vimentin (red), prolyl-4-hydroxylase (P4HB, green) and the nucleus (Hoechst 33342, blue). NHDFad grew as individual cells interspersed in the RAFT™ 3D Collagen scaffold resembling fibroblast growth *in vivo*[1]. **C)** Normal Human Bronchial Epithelial Cells (NHBE, Lonza Cat. No. CC-2540) were seeded on top of RAFT™ 3D Cultures containing normal human Bronchial Smooth Muscle Cells (BSMC, Lonza Cat. No. CC-2576). After 7 days of culture cells were stained for cytokeratin (red) and nuclei were counterstained in blue (Diamidino-2-phenyindole, dilactate (DAPI)). The NHBE formed a dense cell layer on top of RAFT™ Cultures. Scale bar: 100 µm

Figure 3. Efficient Transfection for RAFT™ 3D Cultures

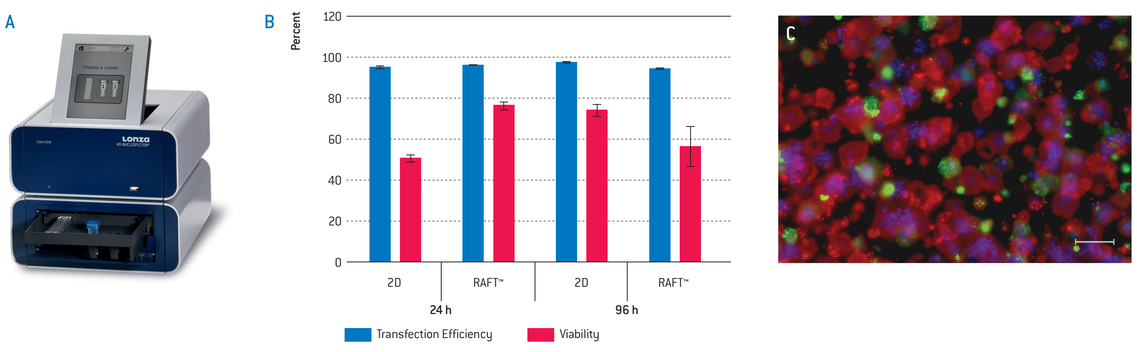

Fig. 3: A) The 4D-Nucleofector™ System with its accessories – the 20 µL Nucleocuvette™ Strip and the 100 µL Nucleocuvette™ Vessel – visible in the X-Unit. **B)** Transfection efficiency and viability of HCT 116 cells in 2D and RAFT™ 3D Cultures. HCT 116 cells were transfected with pmaxGFP™ Vector in 100 µl Nucleocuvette™ Vessels. Cells were seeded in 2D or into RAFT™ 3D Cultures in 96-well cell culture plates. 24 hours and 96 hours post Nucleofection, cells were extracted from the RAFT™ Cultures using 1mg/ml Collagenase (Sigma Aldrich Cat. No. C9722-50MG) and transfection efficiency was analyzed on a FACSCalibur™ (Becton Dickinson). Cell viability was determined using Vialight™ Plus Assay and normalized to untransfected control samples. (n=3) **C)** pmaxGFP™ transfected HCT 116 cells in RAFT™ 3D Cultures 96 hours after transfection. Cells were stained for Vimentin (red) and nuclei were counterstained in blue (Hoechst 33342). Scale bar: 100 µm

Figure 4. Immunocytochemistry of RAFT™ 3D Cultures

A

Step	Time	Reagent
Washing	3 × 15 min	PBS
Fixation	30 min	3.7% PFA in PBS
Quenching	10 min	1mM Tris, 20mM Glycine in PBS
Washing	3 × 15 min	PBS
Permeabilize	4 min	0.1% or 1.0% Triton X-100 in PBS
Washing	3 × 15 min	PBS
1° Antibody	Overnight	in 0.1% or 1.0% Triton X-100, 1.0% BSA in PBS
Washing	3 × 15 min	PBS
2° Antibody	2.5 hrs	in 1.0% BSA in PBS
Washing	3 × 15 min	PBS

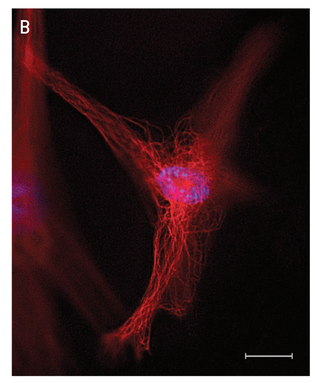

Fig. 4: A) Immunocytochemistry Protocol for RAFT™ 3D Cultures. The concentration of Triton X-100 (Sigma Aldrich) in the permeabilization and 1° antibody step is dependent on cell type. For cells like NHDF that grow as single cells in the RAFT™ Collagen scaffold a concentration of 0.1% Triton X-100 is usually sufficient. For cell types like HCT 116 that form aggregate-like structures in RAFT™ a higher Triton X-100 concentration of 1.0% is recommended. **B)** NHDF were cultured in the RAFT™ System for 11 days. Cells were stained for tubulin (red) and the nucleus (blue). One z-plane location was imaged on a widefield microscope. The image from each channel was deconvolved using the 3D spectral deconvolution software from ImageJ (generalised Tikhonov) and the merge of the two channels is shown here. Scale bar: 20 µm

Figure 5. ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay

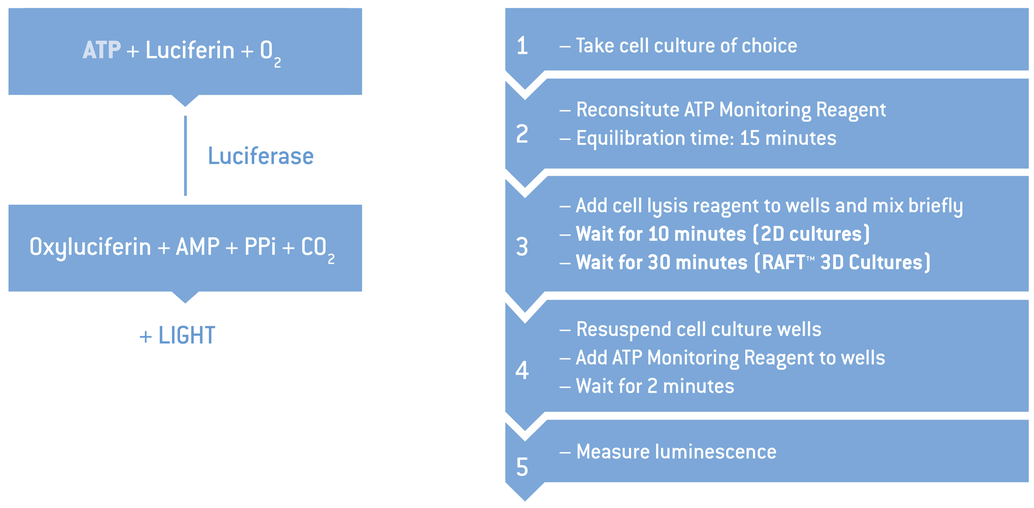

Fig. 5: A) Principle of the ViaLight™ Plus BioAssay. The emitted light that can be detected using suitable luminometers or scintillation counters. **B)** Overview of the ViaLight™ Plus BioAssay Procedure. For RAFT™ 3D Cultures it is recommended to increase the lysis time (Step 3) from 10 minutes to 30 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

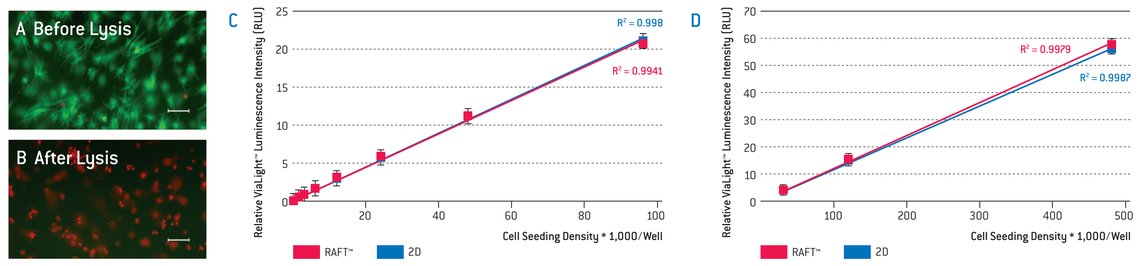

Fig. 6: A, B) NHDFneo are efficiently permeabilized by the Vialight™ Plus BioAssay Lysis Buffer. NHDFneo were cultured in RAFT™ Collagen scaffolds before being stained with Calcein AM for viable cells (green) and Propidium Iodide for dead cells (red). Subsequently cells were exposed to the Vialight™ Plus BioAssay Lysis Buffer while being monitored microscopically. After 10 minutes efficient lysis of cells was observed, indicated by the loss of Calcein AM fluorescence from the cells, while Propidium Iodide stained nuclei became visible. Scale bar: 100 µm. **C, D)** Linear Range of the Vialight™ Plus BioAssay for HCT 116 cells in **C)** 96-well or **D)** 24-well RAFT™ 3D Cultures. Cells were cultured for 2 hours before being analyzed. The mean luminescence is plotted against the cell seeding density. The error bars represent the standard errors.

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 Nucleofection Technology.
- The RAFT™ 3D Culture System can be analyzed using standard cell biology methods like immunocytochemistry or the Lonza Vialight™ Plus Bioassay.

References

1. H.J. Levis, R.A. Brown, J.T. Daniels (2010) Plastic compressed collagen as a biomimetic substrate for human limbal epithelial cell culture. *Biomaterials*, pp. 1–12.