BioResearch

Abstract: 1702

Analyzing Cell Viability in 3D Tissue Models with the ViaLight™ Plus BioAssay

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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 resembling more the *in vivo* environment. Cells can develop cell-cell and cell-extracellular matrix (ECM) interactions in 3D.

While 3D cultures more accurately approach the *in vivo* environment, it might be difficult to analyze cells in 3D. However, this is not always the case. This poster explains how to measure cell viability easily in 3D cell cultures using the ViaLight™ Plus BioAssay. Cell viability was determined in two different 3D cell culture systems – the RAFT™ 3D Cell Culture System and classical spheroid cultures generated in ultra-low attachment plates.

Materials and Methods

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

ViaLight™ Plus BioAssay

The ViaLight™ Plus BioAssay is based on the bioluminescent detection of cellular ATP as a measure of cell viability. The easy two-step, non-radioactive assay protocol was designed to provide robust, quick and sensitive measurements of cell viability and cell proliferation. In addition, the two-step assay protocol allows users to adapt the length of the first lysis step according to the needs of their cell culture system.

Figure 1. ViaLight™ Plus BioAssay

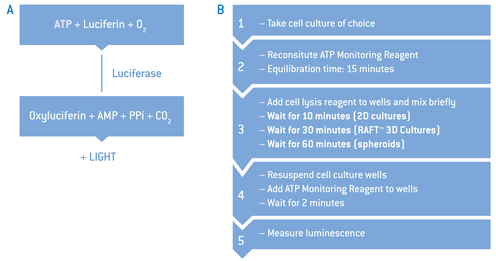

Fig. 1: A) Principle of the ViaLight™ Plus BioAssay. The emitted light that can be detected using suitable luminometers or scintillation counters. **B)** Overview of the ViaLight™ Plus BioAssay Procedure. For RAFT™ 3D cultures, it is recommended to increase the lysis time (Step 3) from 10 minutes to 30 minutes to ensure efficient lysis of the approximately 100–120 µm thick cultures. For spheroid cultures, it is recommended to increase the lysis time (Step 3) to 60 minutes to ensure efficient lysis of spheroids with a diameter of up to 800 µm.

RAFT™ 3D Culture System

The RAFT™ 3D Culture System uses an approximately 120 µm thick collagen matrix which mimics physiological conditions. 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 2. The RAFT™ 3D System Process

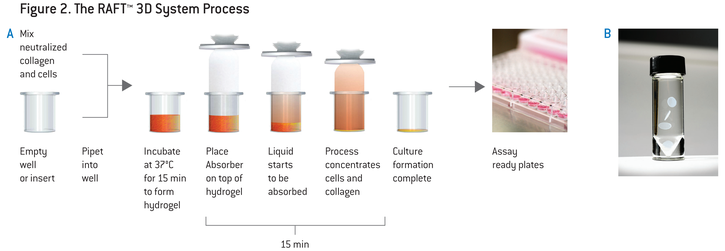

Fig. 2: A) The RAFT™ 3D System Process. Cell containing RAFT™ Cultures are formed within less than one hour. **B)** Floating RAFT™ Cultures.

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

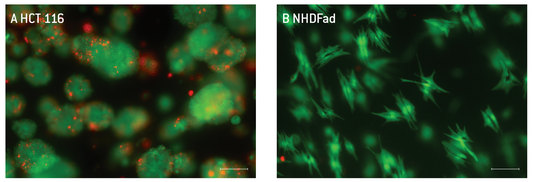

Fig. 3: A) HCT 116 cells were cultured for 6 days in RAFT™ 3D Cultures before being stained with Calcein AM for viable cells (green) and Propidium Iodide to detect dead cells (red). The colon cancer cell line formed tumor-like structures in RAFT™ 3D Cultures. **B)** Neonatal Dermal Fibroblasts (NHDFneo; Lonza Cat. No.: CC-2509)were cultured in RAFT™ for 72 hours before being stained with Calcein AM and Propidium Iodide. NHDFneo grew as individual cells interspersed in the RAFT™ 3D collagen scaffold resembling fibroblast growth *in vivo*[1]. Scale bar: 100 µm

Figure 4. Performance of the ViaLight™ Plus BioAssay in RAFT™ 3D Cultures

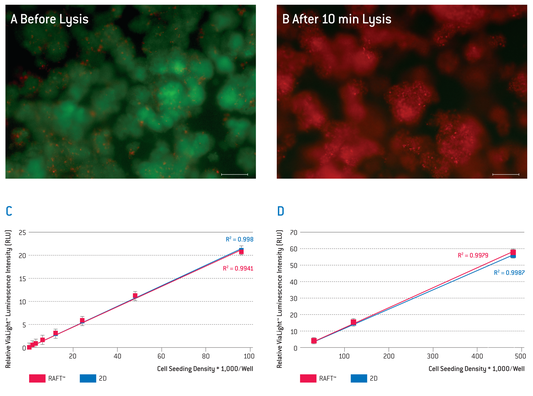

Fig.4: A, B) HCT 116 are efficiently permeabilized by the Vialight™ Plus BioAssay Lysis Buffer. HCT 116 were cultured for 6 days in RAFT™ before being stained with Calcein AM for viable cells (green) and Propidium Iodide for dead cells (red). Subsequently cells were exposed to the ViaLight™ Plus BioAssay 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.

Figure 5. Spheroid Formation of HCT 116 Cells in Ultra-low Attachment Plates

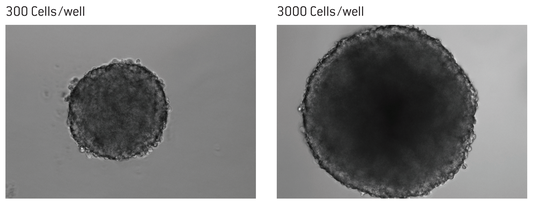

Fig. 5: Spheroid formation of HCT 116 cells in ultra-low attachment plates. The indicated number of cells was seeded into 96-well ultra-low attachment round-bottom microplates (Corning Cat. No.: 7007) and cultured for 72 hours Scale bar: 100 µm

Figure 6. Lysis of HCT-116 Spheroids by ViaLight™ Plus Lysis Buffer

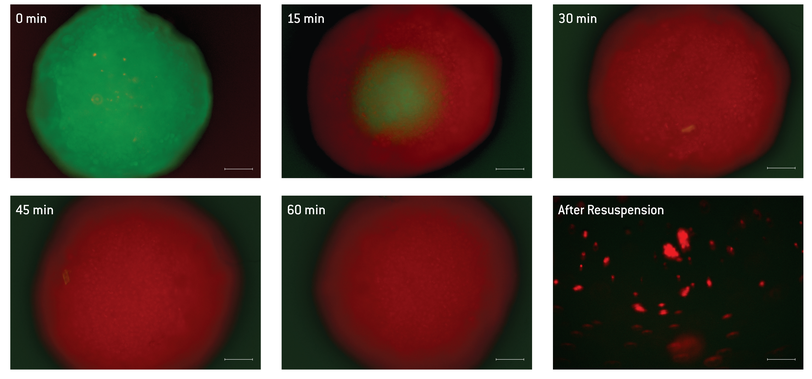

Figure 6: HCT 116 spheroids are efficiently permeabilized by the ViaLight™ Plus BioAssay Lysis Buffer. 1,000 HCT 116 were cultured for 5 days in 96-well ultra-low attachment plates before being stained for 30minutes with Calcein AM for viable cells (green) and Propidium Iodide to detect dead cells (red). Subsequently, 50 µl of ViaLight™ Plus BioAssay Lysis Buffer were added to each well. Efficient lysis of the spheroids was observed after 30 minutes. This is indicated by the loss of Calcein AM fluorescence, while Propidium Iodide stained nuclei became visible. After resuspension, the spheroids dissolve into smaller, Propidium Iodide positive cell clusters. Scale bar: 100 µm

Figure 7. Performance of the ViaLight™ Plus BioAssay for HCT 116 Spheroids

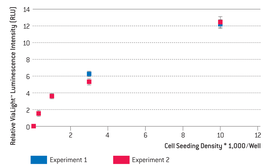

Figure 7: Performance of the ViaLight™ Plus BioAssay for HCT 116 spheroids. In two independent experiments, HCT 116 were seeded in the indicated concentrations in 96-well ultra-low attachment plates and cultured for 3 days at 37°C and 5% CO_2. Subsequently, cell viability was assessed using the ViaLight™ Plus BioAssay with a lysis time of 60 minutes. The mean luminescence recorded for each quadruplicate is plotted against the cell seeding density. The error bars represent the standard errors over each quadruplicate. A linear range of the assay is observed for spheroid diameters of up to 500 µm. For larger spheroids, no linear performance of the ViaLight™ Plus BioAssay could be observed, very likely due to increased cell death in the oxygen- and nutrient-depleted spheroid core.

Conclusions

- The ViaLight™ Plus BioAssay is suitable for monitoring cell viability in RAFT™ 3D Cell Cultures and spheroid cultures.
- By extending the lysis time from 10 minutes to 30 minutes, efficient permeabilization of cells and dissolution of the RAFT™ 3D collagen scaffold can be achieved.
- By extending the lysis time from 10 minutes to 60 minutes, efficient permeabilization and dissolution of spheroids with a diameter of up to 800 µm can be achieved.
- A linear range of the assay is observed for cell seeding densities of up to 96,000 cells per 96-well RAFT 3D Cell Culture and for spheroid diameters of up to 500 µm. For larger spheroids, no linear performance of the ViaLight™ Plus BioAssay could be observed, very likely due to increased cell death in the oxygen- and nutrient-depleted spheroid core.

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.