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Validation of the Cellbox® for Simulated Transport of a Liver derived Cancer Cell Line

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Key Words

HepG2, Portable CO_2 incubator, Live Cell Shipper, Cell Viability, Transport, Shipping.

Goals

To validate the performance of the Cellbox® flight CDI as a CO_2 incubator for cultivating cells under stationary and simulated transport conditions.

Abstract

The quality and viability of biological cells and tissues can be severely impacted by inadequate shipping conditions. To address this issue, Cellbox Solutions GmbH has developed a live cell shipper for transporting cells under laboratory conditions. In this application note the performance of the Cellbox® flight CDI was investigated by comparing it to a standard CO_2 incubator. Experiments performed at the Fraunhofer IME ScreeningPort assessed the ability of the Cellbox® to maintain cultures of HepG2 cells under stationary and simulated transport conditions. Assays testing the viability, confluency and mitochondrial morphology of HepG2 cells confirmed that the Cellbox® provides incubation conditions that are equivalent to a standard CO_2 incubator during stationary and simulated transport conditions.

Introduction

The transport of cells and other biological material, locally and internationally, demands well maintained and documented temperature-controlled logistics solutions (1). Traditionally, cells are cryopreserved before being transported under cryogenic temperatures. Following delivery, cells can either be thawed and recovered or transferred to a freezer for storage. These traditional approaches are however becoming less compatible with the increasingly complex cellular materials used in drug discovery, life science research and healthcare applications. Furthermore, limited access to expensive freezing and thawing equipment, as well as deviation from the standard handling procedures contribute to industry tolerated losses in post-thaw cell viability and quality (2).

To address the aforementioned losses in cell viability and cell quality, Cellbox Solutions GmbH has introduced a live cell shipping incubator with an international flight allowance. By implementing a unique patented system, the Cellbox® flight CDI uses dry ice pellets for conditioning of the internal incubation chamber with CO_2, instead of the industry standard gas cylinders. At the heart of this technology is the natural process of dry ice sublimation, which is harnessed to generate a reservoir of gaseous CO_2.

In this application note HepG2 cells, an adherent cell line of hepatocytes with hepatocellular carcinoma, were cultivated in a Cellbox® flight CDI under stationary and simulated transport conditions (3). By assessing the viability, confluency and mitochondrial morphology of the cells, it could be shown that cells incubated in the Cellbox®, even during simulated transport, are comparable to those grown in a standard CO_2 incubator.

Experimental Setup and Procedures

Equipment

- Cellbox® flight CDI (5 % CO_2, 37 °C)
- Thermo Fisher Scientific Cytomat 6000 (5 % CO_2, 37 °C)
- Infors HT Multitron Shaker (50 RPM)
- Labcyte Echo Liquid Handler
- PerkinElmer EnSight Multimode Plate Reader
- PerkinElmer EnSpire Multimode Plate Reader
- PerkinElmer Opera High Content Imaging System with PerkinElmer Columbus software
- Vortex Shaker
- Centrifuge

Consumables and Reagents

- HepG2 Cells (DSMZ no.: ACC 180)
- Dry ice nuggets, 10 mm diameter
- Collagen 1, rat tail
- Ham's F12 medium, supplemented with 2 mM L-glutamine and 10 % Fetal Bovine Serum
- Trypsin-EDTA (0.05 %) in DPBS
- Dulbecco's phosphate-buffered saline (DPBS), without Calcium and Magnesium
- Promega CellTiter-Glo® Luminescent Cell Viability Assay
- Greiner Bio-One CELLSTAR® 384-well plates, white
- Valinomycin - potassium ionophore, alters mitochondrial function (4)
- DMSO

- Rifampicin - hepatotoxic antibiotic (5)
- 4titude® Moisture barrier Seals
- 4titude® 384-well clear bottom plates
- Thermo Fisher Scientific MitoTracker® Red CMXRos

Workflow

HepG2 cells were seeded in 384-well plates and cultivated in the Cellbox® under stationary (Fig.1A) and simulated transport (Fig.1B) conditions for 48 hours. The cells were incubated in a standard cell incubator for 48 hours as a control (Fig.1C). After the incubation period, HepG2 cells from each of the conditions were assessed for viability, confluency and mitochondrial morphology.

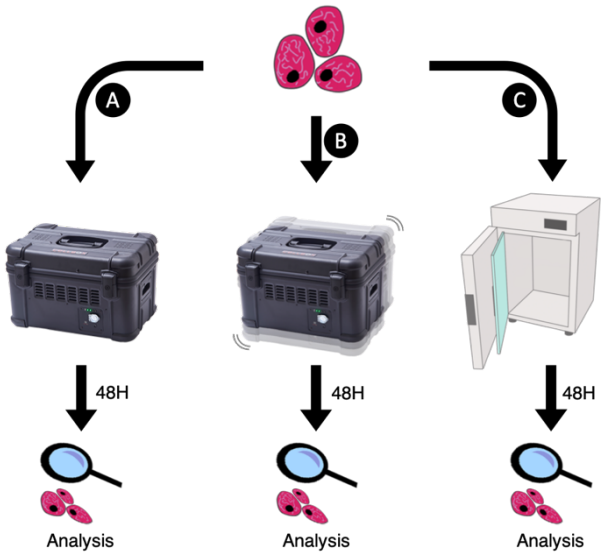

Figure 1. Experimental workflow for investigating HepG2 incubation under stationary and simulated transport conditions in the Cellbox® flight CDI. HepG2 cells were seeded in 384-well plate format and cultivated for 48 hours (A) in the Cellbox® under stationary conditions, (B) in the Cellbox® under simulated transport conditions on a rotary shaker and (C) in a standard cell incubator. Following incubation, the viability of the cells was assessed.

Cellbox® flight CDI handling procedure

Prior to the experiments the Cellbox® was fully charged and the temperature set to 37 °C. Next, the dry ice container was filled with dry ice nuggets to provide a CO_2 supply and the CO_2 concentration was set to 5 %. Cells were then transferred to the Cellbox® for stationary incubation (Fig.1A). The Cellbox® was continuously charged and the dry ice reservoir refilled once every 24 hours.
Alternatively, cells were transferred to the Cellbox® and the device disconnected from the power supply before being placed on a shaker at 50 rpm, to simulate transport (Fig1.B). Shaking was always performed overnight and the Cellbox® was recharged during the day (remained stationary while charging). The dry ice reservoir was refilled once every 24 hours.

HepG2 Cell Culture Protocol

Assay plates were coated with collagen (5 µg/cm^2) before use by means of a standard coating protocol. HepG2 cells were thawed and cultivated in Ham's F12 medium supplemented with 2 mM L-glutamine and 10 % fetal bovine serum, before being harvested using Trypsin/EDTA. The collected cells were counted and seeded at a defined cell number into the assay plates (20 µl/well). Following overnight incubation in a standard cell incubator, the cells were used as described in the workflow and subsequent assays.

CellTiter-Glo® Assay

HepG2 cells were seeded in white 384-well plates (Cellstar, greiner bio-one) at 2500 cells/well. After overnight incubation in a cell incubator, the compounds Valinomycin, Rifampicin and DMSO (control) were added to the assay plates using the Echo Liquid Handler (Labcyte). Wells with untreated cells were also included for comparison. After the compounds were added, the plates were sealed with Moisture Barrier Seals (4titude) and placed in in the Cellbox® (Fig.1A and Fig 1B), or a standard cell incubator (Fig.1C). After 48 hours of incubation, assay plates were removed from the Cellbox® and the cell incubator and equilibrated to room temperature for 30 min. In parallel to the previous step, the CellTiter-Glo reagent was prepared according to the manufacturer's protocol. 10 µl/well of reagent were dispensed into the assay plates. Plates were placed for 30 s on a shaker and centrifuged shortly (pulse, 1000 rpm). After 10 min of incubation in the dark, the luminescence was detected on the EnSpire plate reader.

Confluency detection

Cells were seeded in black 384-well clear bottom plates and incubated overnight. Confluency was then detected using the EnSight plate reader before being transferred to the appropriate conditions for incubation as set forth in the experimental workflow (Fig.1). After 48 hours the confluency was measured again.

Mitochondria Staining

Cells were seeded in black 384-well clear bottom plates (4titude) and incubated in the Cellbox® flight CDI or a cell incubator. After 48 hours of incubation the cells were treated with 0.5 % (v/v) DMSO as a control. After 1.5 hours (HepG2) incubation at 37 °C and 5 % CO_2, 10 µl of MitoTracker® Red CMXRos (final concentration 50 nM in prewarmed cell culture media was added to each well and the cells were incubated for an additional 45 min at 37 °C and 5 % CO_2. MitoTracker® Red CMXRos uptake was detected using the Opera or Operetta High Content Imaging System (PerkinElmer). The obtained images were analysed using Columbus software (PerkinElmer).

Results and Discussion

Cell-based assays commonly used in drug discovery, healthcare and life science workflows rely on the quality and viability of the input-material. In order to gain the best results from these assays, it is of paramount importance to optimize all upstream handling processes. One of these processes, which is continuously being improved, is the transport of cells, cell cultures and other biological material, both over short and long distances.

Transport solutions are available from commercial partners at ambient to cryogenic temperatures, with

each solution offering distinct advantages and disadvantages. Traditionally cryogenic shipping solutions are favoured, but due to a lack of optimization and compatibility consignees can expect cell losses of up to 70 % for certain cell types (2).

As an alternative to the standard shipping solutions, Cellbox® live cell shippers can be used to transport cells under laboratory conditions in formats that are compatible with immediate processing by automated platforms. By using three separate approaches (Fig.1) the Cellbox® was validated for maintaining live cell cultures.

CellTiter-Glo® Assay

A CellTiter-Glo assay was performed to ensure that the viability of HepG2 cells was unaffected by incubation and simulated transport in the Cellbox®. This assay functions on the principle of ATP detection and is a widely accepted marker for the integrity of the cell membrane (6).

Untreated cells and cells grown in the presence of 0.5 % DMSO (vehicle control) showed comparable viability when cultured in the Cellbox® under all tested conditions (Fig.2A & 2B).

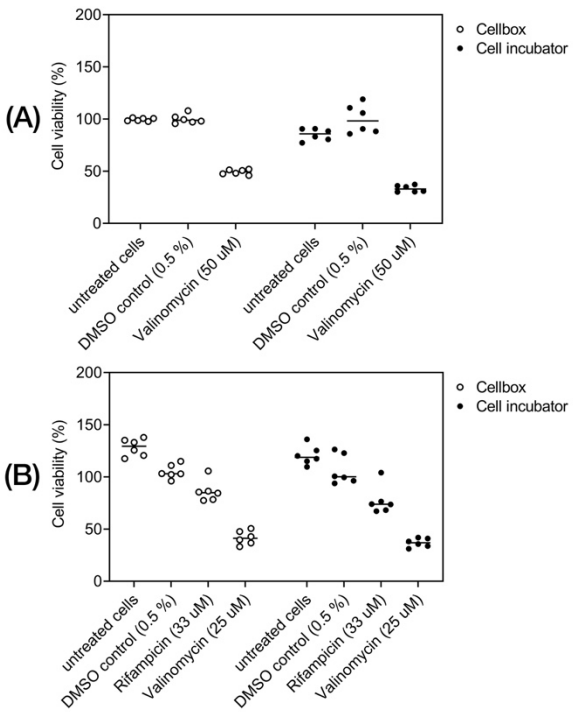

Figure 2. Viability of HepG2 cells incubated in the Cellbox® flight CDI under stationary and simulated transport conditions. (A) Incubation of HepG2 cells in a stationary Cellbox® or cell incubator for 48h, treatment with 0.5 % (v/v) DMSO or 50 mM Valinomycin (6 technical replicates, median displayed). **(B)** Incubation of HepG2 cells in a Cellbox® on a shaker or cell incubator for 48 h, treatment with 0.5 % (v/v) DMSO, 25 mM Valinomycin or 33 µM Rifampicin (6 technical replicates, median displayed).

As expected, a decrease in cell viability was seen after 48 hours in the presence of Valinomycin under all tested conditions (Fig.2A & 2B). Similarly, Rifampicin also decreased cell viability of cells grown during simulated transport in the Cellbox and in a standard incubator (Fig. 2B).

Confluency detection

Confluency is a commonly used term that serves as an indicator for cellular growth and the coverage of the growth surface by cells. When culturing adherent cells, it is vital to avoid detachment and disrupt the culture. To understand the influence of transport on cell adherence, the confluency of HepG2 cells was assessed before and after 48 hours of incubation. Incubation was performed in a Cellbox® under stationary conditions, in a Cellbox® under simulated transport conditions and in a standard incubator (Fig.1).

Confluency values of the HepG2 cell cultures showed that no significant variation could be observed when comparing cells grown in the Cellbox® under stationary conditions to those grown in a standard incubator (Fig. 3A). Furthermore, simulated transport conditions did not lead to any noticeable cell detachment when compared to growth in a standard incubator (Fig.3B).

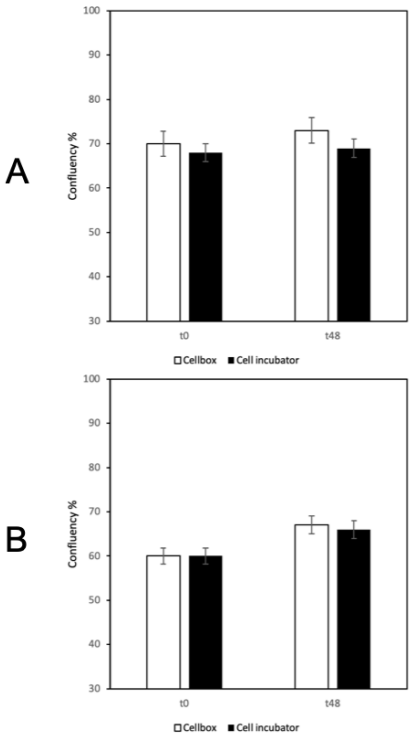

Figure 3. Confluency (%) detection of HepG2 cells incubated in the Cellbox® flight CDI under stationary and simulated transport conditions. (A) Confluency was detected before and after 48h of incubation in a stationary Cellbox® and a cell incubator. **(B)** Confluency was detected before and after 48h of incubation under simulated transport conditions in a Cellbox® and a cell incubator. Shown are the mean ± SD, n=66 wells.

Mitochondrial Function Assay

Mitochondrial function is a key indicator of the health of cells and losses in mitochondrial membrane potential are known to trigger cell death via apoptosis (7). To exclude the possibility that stationary incubation and simulated transport in the Cellbox® may result in apoptosis, a high content imaging assay based on MitoTracker® Red CMXros mitochondrial staining was carried out. This fluorescent dye stains healthy mitochondria the accumulation thereof is dependent on the membrane potential (8).

By assessing the granularity of the MitoTracker® signal it was possible to show that stationary incubation (Fig.4A) and simulated transport (Fig.4B) in the Cellbox® does not affect the mitochondrial function of HepG2 cells.

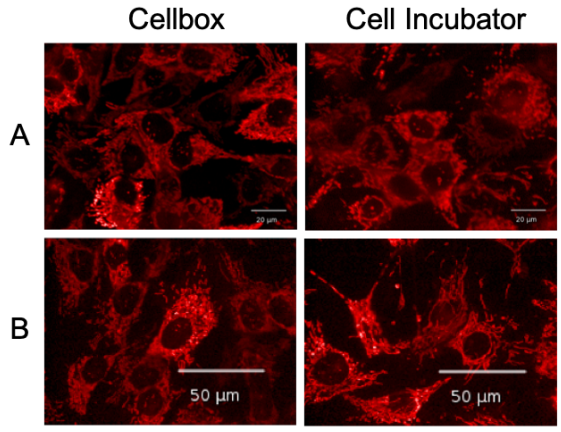

Figure 4. Mitochondrial function assay of HepG2 cells incubated in the Cellbox® flight CDI under stationary and simulated transport conditions. MitoTracker® Red CMXRos staining of the mitochondria of HepG2 cells treated with 0.5 % (v/v) DMSO. **(A)** HepG2 cells incubated in a stationary Cellbox® (left) and a cell incubator (right). Scale bar represents 20 µm **(B)** HepG2 cells incubated in a Cellbox® under simulated transport conditions (left) and a cell incubator (right), Scale bar represents 50 µm.

Conclusion

The work presented in this application note demonstrates that the Cellbox® flight CDI offers incubation conditions that are comparable to commercially available laboratory incubators. Incubation of HepG2 cells in the Cellbox® showed no negative influence on the cell viability, cell detachment and mitochondrial function of the cultures. The data also indicates that simulated transport conditions have no harmful effects on HepG2 cells.

As the complexity of cell and tissue cultures continue to increase, the Cellbox® offers a viable alternative to the conventional methods that are used to ship and transport cells.

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