An automated system for a stable and reproducible long-term cultivation of epithelial cells at the air-liquid interface

The CULTEX® LTC module

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Introduction

In vitro approaches, especially cell culture techniques, have gained more and more importance in toxicology during the last decades, due to the ongoing progress in the development of novel technologies and the increasing call for alternatives to animal testing. One major requirement for the reliability of toxicological data is their reproducibility. For that purpose, identical cell cultures are required for repeatable experiments. However, the handling of cell cultures is laborious and vulnerable to person-specific variations as well as pipetting errors. Thus, automated cell cultivation systems are highly desirable for in vitro toxicology. There are various automated systems that are specifically designed to fulfill particular requirements, ranging from different bioreactors up to sophisticated modular working stations.

Today, cell cultures that reflect in vivo conditions in the best possible manner are often used for toxicological studies. The lung, skin and intestinal epithelium are target organs of many drugs and pollutants and are thus the subject of most toxicological studies. The differentiation of epithelial cells in vitro is achieved by culturing them at the air-liquid interface (ALI). The CULTEX® Long Term Cultivation (LTC) system was specifically designed for the computer-controlled cultivation of cells grown on commercial cell culture inserts under ALI conditions. The system allows a continuous as well as an intermittent medium supply and the medium level is controlled by an ultrasonic sensor. The results demonstrate that the CULTEX® LTC module enables the long-term cultivation of epithelial cells under stable and reproducible conditions and can thus highly enhance the reliability of toxicological studies.

Materials and Methods

CULTEX® LTC unit:

The LTC equipment is specifically designed to provide ALI cell cultures with culture medium over a longer time period (e.g. 3–6 weeks). The medium change interval and volume of medium renewal as well as the frequency of medium change (interval and volume) are programmable. The medium is balanced continuously by reheating the water. All parts contacting the medium can be removed individually and are autoclavable at 121 °C.

Control of the medium level is enabled by an ultrasonic pulse-echo sensor. The pumps are located inside the control unit in a sliding cassette, the sample uptake module and the control unit are connected to the network (230 Vac) via cables. The module package consists of the incubator module, the supply module, the LH module, the control unit and the sample uptake module. The LH module includes the ultrasonic pulse-echo sensor, the supply module and the control unit.

A549 cultures were immediately placed into the CULTEX® LTC-C and cultivated for varying culture periods.

Preparation of cell cultures:

The A549 cells were expanded in 75 cm² cell culture flasks with DMEM (including L-glutamine, sodium pyruvate) supplemented with 10% FBS and 0.05% gentamicin. After passaging, the cells were seeded on cell culture inserts (12 well format) with a density of approximately 3 × 10⁴/cm² and cultivated under submerged conditions with their respective growth medium (DMEM) until reaching 100% confluency. Afterwards, the air-liquid interface was established by removing the apical medium from the insert housing and replacing the growth medium in the basal chamber by the differentiation medium [BEBM (Lonza)/DMEM (1:1) supplemented with SingleQuots™ Lonza, without retinoic acid, 100 nM retinoid acid and 2% Ultroser G (Pall Corporation)]. The A549 cultures were immediately placed into the CULTEX® LTC-C and cultivated for varying culture periods.

Conclusions and Outlook

The investigations demonstrated:

- Cells grown under long term conditions in the CULTEX® LTC-C exhibited a homogenous growth independent on the insertion position within the support plate.
- In comparison to the control cultures which were grown under conventional culture conditions (manual medium exchange every 36 hours), it became obvious that the circulation of the medium induced a considerable proliferation activity in A549 cells, reflecting their cancerous origin.
- The cell growth of A549 cultures can be influenced by the frequency of the medium renewal and circulation of the culture.

The advantages of such an experimental approach can be summarized as follows:

- A desired number of ALI cultures can be grown under comparable and reproducible culture conditions – independent on interferency by manual handling procedures.
- The provision of constant culture conditions led to a much more stable and reproducible data collection. The influencing factors for growth and differentiation can be defined in order to select the relevant exposure conditions with regard to the susceptibility of the cells.

Results

Functionality of the long-term cultivation device & procedure was analyzed by using A549 cells.

Cell growth was studied dependent on (1) cultivation time, (2) position in the support plate (Figure 5), (3) influence of the sensor and (4) reproducibility (3 independent experiments).

Experimental conditions: Medium renewal: 36 hours, circulation interval: 30 minutes, circulation frequency: 2 every 30 minutes

NHBE cells (passage 2) were cultivated in BEGM Lonza. The cell culture inserts were inoculated with 1.5 × 10⁵ cells/cm² and the cultures were cultivated under submerged conditions with BEGM. When reaching 100% confluency, the ALI cultivation was started (same culture conditions as for A549 cultures). After 10 days under ALI conditions, the NHBE cultures were placed into the CULTEX® LTC-C and cultivated for varying culture periods.

The experimental conditions are given in the results section. For evaluation of the cultures, the cell cultures were fixed with 10% formaldehyde and the inserts membranes were embedded in paraffin. Histological sections of 5–7 µm were produced and stained with hematoxylin and eosin (H&E).

Figure 1: Overview on the CULTEX® Long Term Cultivation Continuous (LTC-C) unit for culturing cell cultures growing at the air-liquid interface (ALI). The incubator module consists of the cover plate, sample uptake module, air bubble separator and supply module. All components except the supply module are autoclavable. ALI cell culture inserts (12 wells format; e.g. Cultiwell® or Falcon®), can be handled at the same time. The CULTEX® LTC-C is connected to a control unit (Figure 3), regulating the time intervals and volume of medium renewal as well as the frequency of medium circulation. Before entering the sample uptake module, the fresh culture medium passes the air bubble separator in order to eliminate air bubbles. Control of the medium levels is enabled by an ultrasonic pulse-echo sensor.

Figure 2: The control unit which is located outside the incubator includes a programmable logic controller (PLC) and pumps for the supply and renewal of medium. The control unit is further equipped with an integrated web server. Visualization of the procedure and measurement data is carried out by a web browser, e.g. via a laptop computer. The pumps are located inside the control unit in a sliding cassette. The Liquid Handling (LH) module (Figure 3), where they are easily accessible for maintenance and sterilization.

Figure 3: The main components of the LH module are two peristaltic pumps (supply and removal pump), the Heat exchanger, and the leakage sensor. The leakage sensor recognizes the leaking fluid in the case of an error and triggers a relevant error message. The Heat exchanger preheats the culture medium to about 37 °C before it is conducted to the incubator module. The right figure shows the heat exchanger (with opened top) which is filled with water. During operation, the heating cartridge first heats the water to 37 °C and then transmits the heat to the culture medium, which is conducted through the immovably tubing. A magnetic stirrer circulates the water and this ensures optimal heat transport between the heating cassette and the liquid exchange housing. The heat exchange transferred to the medium is balanced continuously by reheating the water. All parts contacting the medium can be removed individually and are autoclavable at 121 °C.

Figure 4: The CULTEX® LTC-C-operation. The incubator module is placed into the incubator, allowing the cultivation under physiological conditions. The control unit as well as the vessel for culture medium and waste are located outside the incubator.

Figure 5: Distribution scheme for the inserts within the support plate; supply uptake module.

Figure 6: Growth of A549 cells on cell culture inserts with a pore size of 0.1 µm in the LTC-C module depends on the cultivation time. Samples were taken at different time points from the inner and outer circle.

Figure 7: NHBE cells cultivated in the LTC-C for 18 days.

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