AUTOMATION OF CELL-BASED ASSAYS USING THE INHECO SCILA

Application Note

Lena Schober, Richard Rösch, Moriz Walter, Andrea Traube, Fraunhofer-Institut für Produktionstechnik und Automatisierung IPA, Nobelstrasse 12, 70569 Stuttgart, Germany

Martin Rotter, Herwig Angst, Martin Gajewski, Andreas Scholle, Christian George, INHECO GmbH, Fraunhoferstrasse 11, 82152 Martinsried, Germany

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Figure 1: SCILA with one open drawer.

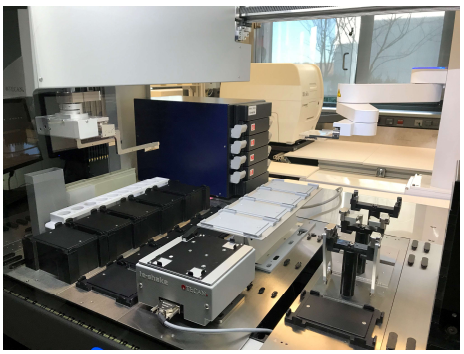


Figure 2: SCILA integrated in automated platform.

INTRODUCTION

Incubation in a controlled environment is one of the key steps in automated cell culture processes. The INHECO SCILA (**S**iLA-based **C**ell **I**ncubator for **L**ab **A**utomation, Fig. 1) can be seamlessly integrated in various liquid handling platforms using the SiLA communication standard. SCILA is designed for easy maintenance and service and modular scaling up to accommodate higher plate numbers.

Within a nICLAS reference project at Fraunhofer IPA the SCILA was validated for automated cell culture and cellular assays by integration into an automated workflow.

ASSAY SET UP

First, cell proliferation assays were performed to demonstrate the functionality of SCILA for cell expansion. Therefore, different cell types (HEK293, HMSC.BM) were cultivated in 6-well plates over a culture period of three weeks and analyzed in regular intervals. Cellular morphology as well as confluence degree was documented microscopically. By reaching 80% confluency cells were passaged and cell number and viability was assessed. As a reference, the same culture set-up was performed in a stand-alone incubator (Heracell™ VIOS 250i, Thermo Scientific™).

Secondly, as a typical application set-up, an automated cell-based assay was executed by using the SCILA in a workflow together with a robotic arm (Precise Automation) to connect the incubator with a liquid handling system (Fluent®, TECAN) and the analysis instruments (Cytation™ 5, BioTek; GloMax® Discover, Promega, Fig. 2).

HEK293 cells were seeded in 96-well plates and treated with 0.5-20% DMSO. After 24hours cell viability was assessed using CellTiter-Glo® reagent and luminescent measurement. As a reference, the same culture set-up was performed in a stand-alone incubator.

Finally, a cleaning and disinfection protocol was established and tested.

RESULTS

Excellent technical performance for temperature and humidity

Temperature and humidity are critical parameters in cell culture processes. Therefore, the initialization process, the uniformity within all 4 culture plate positions and recovery time are key factors to ensure excellent stable culture conditions within the SCILA instrument.

Initialization process

In a normal lab environment, the specified humidity threshold of >85% relative humidity inside the SCILA is reached after ~25 min and the target air temperature of 37°C ±1°C is reached after around 19 minutes (see Fig. 3, Start temperature 22°C, with water prefilled (22°C), without CO_2, measured in air with 2 temperature sensors in drawer 2).

Uniformity

In steady state the temperature profiles show a high uniformity in the incubation chamber with a maximum variation of 0.5 K between the 4 plate positions (Fig. 4). Note, that the temperature gradient within the chamber from plate position 1 to 4 is designed to generate a humidity ventilation circle. A fan is therefore not needed. Temperature uniformity in a given plate position is 0.3 K at 37°C.

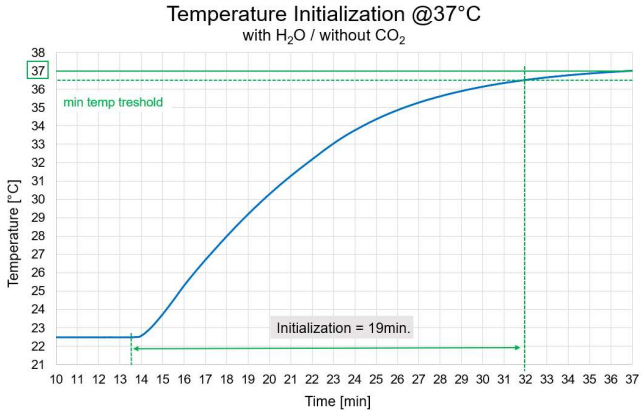


Figure 3

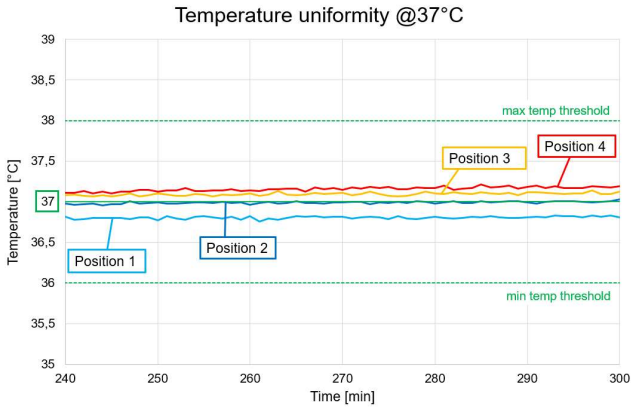


Figure 4

Figure 3 + 4: Graphs show at the x-axis the incubation time [minutes] and at the y-axis the incubation temperature [°C]. In air measurement (Fig. 3) was performed with 2 sensors positioned in drawer 2 (average is shown in Fig. 3). For uniformity measurement (Fig. 4) INHECO Measurement Plates (IMP) -one in each drawer- were used. The different plate positions are numbered, and position is from bottom (Position 1) to top (Position 4). The average value is shown for each drawer gained from 10 sensors distributed in the IMP.

Temperature recovery after drawer opening

After subsequently opening each of the 4 drawers for 10 sec, the recovery time was measured in air (empty incubator, Fig. 5) as well as with 4 IMPs - INHECO Measurement Plates – which have a similar thermal capacity compared to a typical filled cell culture plate (Fig. 6).

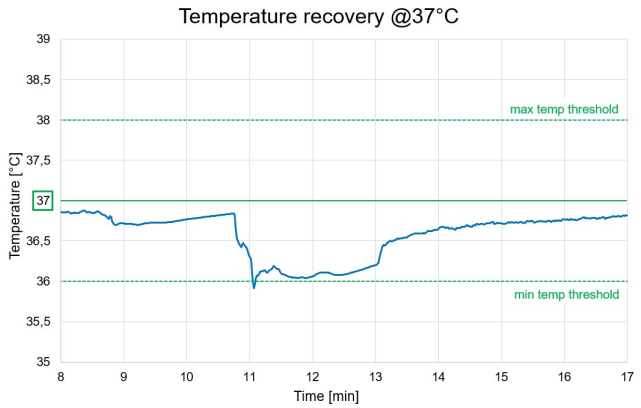


Figure 5

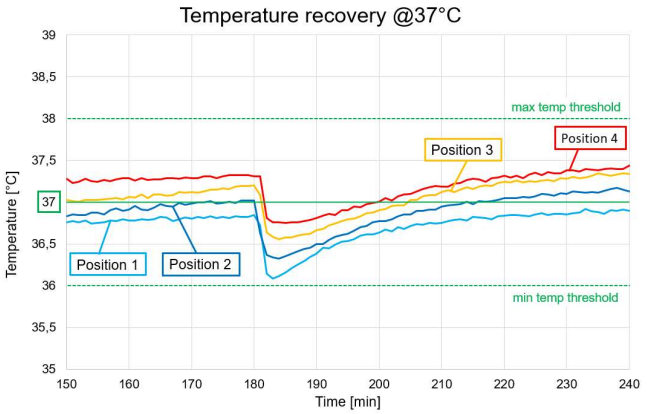


Figure 6

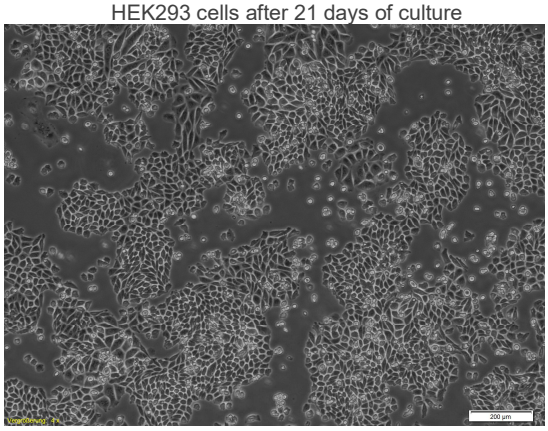
Figure 5 + 6: Graphs show at the x-axis the incubation time [minutes] and at the y-axis the incubation temperature [°C]. Temperature recovery was measured either in air (Fig. 5) with 2 sensors positioned in drawer 2 (average is shown in Fig. 5); or with 4 IMPs -one in each drawer- (Fig. 6). The different plate positions are numbered, and position is from bottom (Position 1) to top (Position 4). The average value is shown for each drawer gained from 10 sensors distributed in the IMP.

In both test scenarios the SCILA design ensures that the temperature always stays in the specified range of 37°C +/- 1 K. No critical overheating occurred while reaching the temperature equilibrium. Cooling down of the plates due to unloading/loading and respective recovery times as observed in manual incubators are not present in the SCILA, guaranteeing optimal culture conditions for the plates that stay in the incubator.

State of the art cell culturing with SCILA

Cell expansion showed similar results in the SCILA compared to the reference in a standard stand-alone incubator at all analysis steps. HEK293 as well as the sensitive HMSC.BM showed typical cell morphology and a high viability of over 80% during several sub-culturing steps over 3 weeks (Fig. 7 + 8). Additionally, the doubling times for the HEK293 cells were comparable between the reference incubator and the SCILA over the whole culturing period; The results are in line with literature data [1,2]. The doubling times for the HMSC.BM were comparable in both systems as well and in line with literature data [3].

HEK293 cells after 21 days of culture



HMSC.BM cells after 21 days of culture

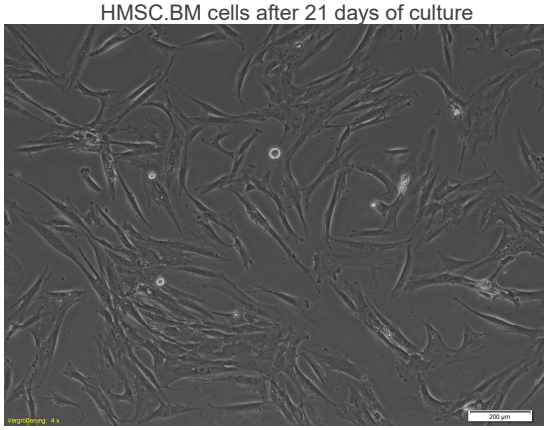
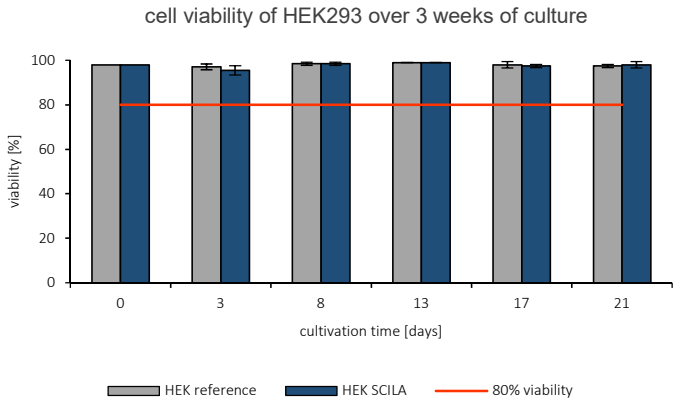


Figure 7



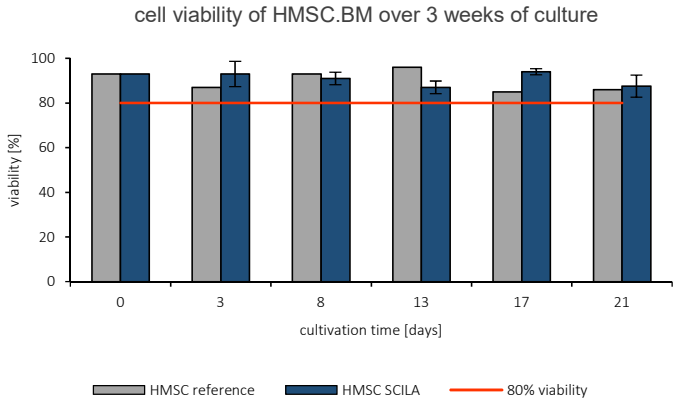


Figure 8

Figure 7 +8: Microscopic images of cell morphology of HEK cells and HMSC.BM in the SCILA after 21 days of culture, respectively (Fig. 7). Graphs show at the x-axis the cultivation time [days] and at the y-axis the cell viability [%] (Fig. 8). Grey bars: reference stand-alone incubator; blue bars: SCILA. Two 6-well plates for each cell type were processed in SCILA; One 6-well plate for each cell type were processed as reference. Starting viability for each cell type is depicted at t=0.

Cleaning and disinfection protocol

The SCILA was set into maintenance mode for cleaning, drawers and door were removed and the SCILA was left open 2 h to contaminate the instrument with room air. After removal of water, the device was cleaned with mild soapy water and afterwards disinfected by wiping with 70 % Ethanol 3 times. 30 min later the device was assembled and put into operation. Sterility was proven by agar contact plates. Samples were taken before and after the cleaning and disinfection on critical control points like bottom, back wall, drawer, front door and insulation rubbers. Contact agar plates were incubated afterwards for 14 days at 30°C showing no growth at all after the described cleaning process in both instruments, the reference incubator and the SCILA. In contrast, samples taken before cleaning and disinfection did show microbe growth as a positive control. In addition, 6-well agar plates were incubated without lid in the cleaned and disinfected SCILA for 14 days. No contamination could be observed.

Automation of cellular assays is comparable to manual execution

With CellTiter-Glo® Luminescent Cell Viability Assay the successful automated execution of a cell-based assay could be demonstrated as outlined in Figure 9. The survival rate of the cells was comparable to the manually processed reference samples (Fig. 10) and in line with literature data. While concentrations of 0.5 and 1% DMSO did not harm the cells (RLU comparable to UTC), with increasing concentration (5% DMSO) the RLU and thus survival dropped down [4, 5].

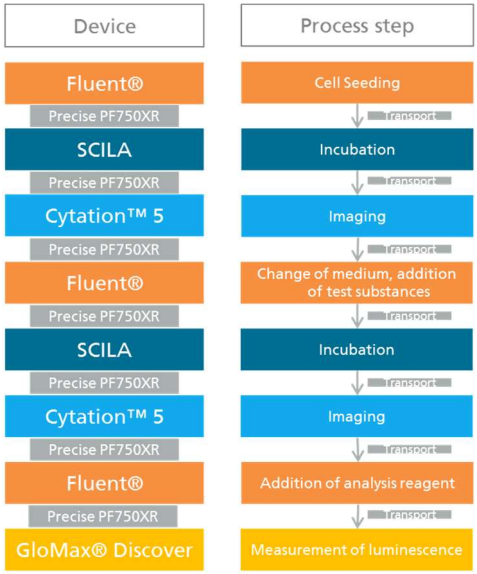


Figure 9

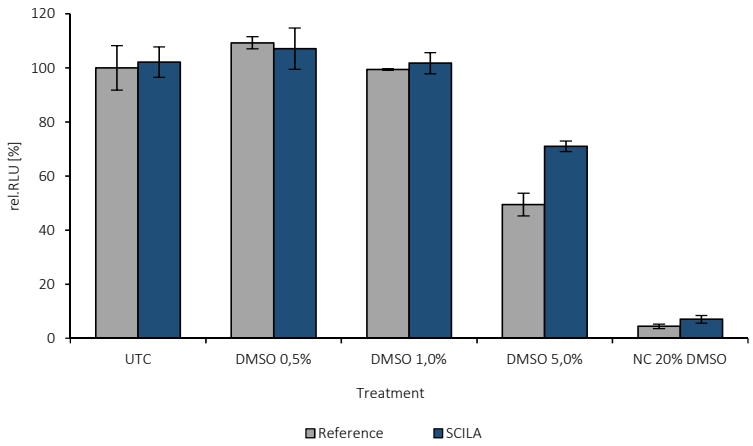


Figure 10

Figure 9 +10: Scheme of automatic workflow with integrated devices and related process steps (Fig. 9). Graph shows at the x-axis the treatment conditions and at the y-axis Relative Luminescence Units (RLU) measured with CellTiter-Glo® Luminescent Cell Viability Assay (Fig. 10). UTC: Untreated cell control (set at 100%), DMSO: treatment with concentration of 1-20%. NC: negative control. Grey bars: reference stand-alone incubator; blue bars: SCILA. 6 wells within 4 different plates were processed for each treatment in SCILA; 6 wells for each treatment within one plate were processed as reference.

SUMMARY

The SCILA from INHECO GmbH is a smart and reliable system to incubate cells in an automated infrastructure. The extremely compact system even allows integration inside many liquid handling systems. It is easy to integrate the system via the SiLA communication standard into a process management software (Lab Automation Control Suite, LACS). The functionality for cell culture could be demonstrated for the HEK293 cell line as well as for the sensitive human bone marrow derived mesenchymal stem cells (HMSC.BM). Furthermore, the automated execution of a cellular assay could be demonstrated. A cleaning and disinfection protocol was successfully proven. All these results highlight the suitability of the SCILA for the automated processing of cell-based assays.

„SCILA is an extremely compact system which is pretty easy to integrate & well applicable for automated cell culture"

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