Disturbance-free, Smart Monitoring of Cell Cuture Growth Using CITSens Bio

In-situ Glucose Measurement During Fibroblast Growth in T-Flasks: Realtime, Unattended, Wireless

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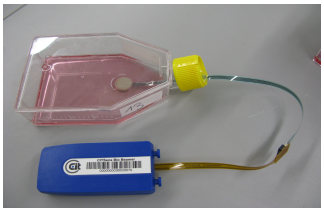
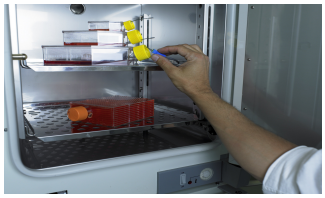
Introduction

C-CIT Sensors is a pioneer in the development of enzyme based sensors for the in-situ measurement of substrates and metabolites in cell cultures. It has been shown that CITSens Bio combines the sensitivity and precision of standard offline technologies applied to quantify the glucose concentration in media with the ability to measure substrates such as glucose in-situ and in realtime. Which in consequence eliminates the need for frequent sample preparation steps and thereby reduces the occurence of artefacts, the risk of contamination, and the cost of labor *(Novel Single-Use Sensors for Online Measurement of Glucose, I. Bauer et al., BioProcess International 10(8), September 2012, p. 56-60)*.

CITSens Bio consists of a sensor built into the flask cap, the data logging device acting as a data beamer, the receiver and PC containing the software and standalone server capacity. Direct measurement of current (nA) data is transferred wireless to the server and can be viewed using C-CIT Sensors' PC or any PC using a web browser. The company's software acts as the interface displaying the evolution of the glucose concentration.



Picture 1: CITSens Bio, Insitu Glucose Sensor built into a T-flask and applied to monitoring the growth of fibroblasts



Picture 2: Disturbance-free cultivation, T-flasks with CITSens Bio placed into an incubator for smart, intervention-free monitoring of cell growth over a time span of 6 d

Goals of the Study:

To show the use of CITSens Bio as a monitoring device of changing glucose concentrations in undisturbed batch cultures of adherent human primary fibroblasts over several days at a starting glucose concentration of 1 g/l. To proof the efficacy of CITSens Bio as a quantitative tool enabling the detection of altered glucose consumption kinetics due to differences in inoculum size: demonstrate the correlation between cell number, the degree of confluency and glucose level.

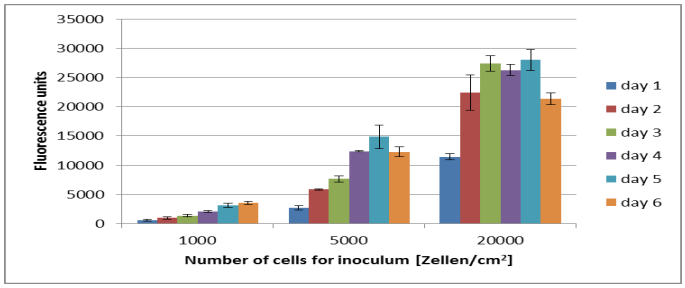
Materials and Methods

3 different numbers of cells were seeded in duplicats into T-75 flasks containing 20 ml medium. After calibration of sensors at 1 g/l glucose, cell growth and glucose concentration were measured over a total time span of 6 d. Initial cell concentrations were 1'000, 5'000, and 20'000 cells/cm^2 respectively. The cells grew free from any intervention and disturbance (closed incubator door). No further calibration of the sensors was performed. Every 20'' a data point was captured. There were only 2 instances of intervention, the first one on day 3 and the second one at the end of the experiment on day 6 (taking pictures using inversion microscopy (Axio Vert 25; Zeiss) and testing of cell viability applying PrestoBlue®).

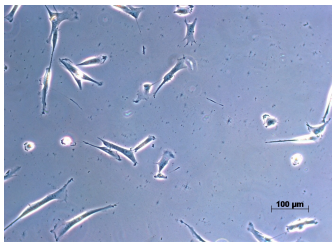
Results

Viability of cells with different seeding densities was measured once a day in the incubator with normal operating state (picture 9). In case of the high seeding density, the cells grew exponentially right from the beginning and maximal cell number was reached after 3 d correlating with a depletion of glucose (picture 8) as well as with full confluency (picture 7). Cells with a seeding density of 1'000 cells/cm^2 remained in lag-phase during time of analysis (pictures 9, 3 and 4). Cells with seeding density of 5'000 cells/cm^2 grew well after a short lag-phase of approx. 24 h and the culture's viability increased till day 5 (pictures 9, 5 and 6).

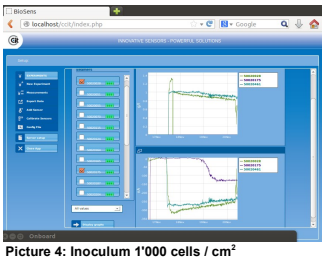
Odd numbers of pictures on the right are showing inverted microscope pictures after 3 days of cultivation. A clear correlation between the degree of confluency after day 3 and the strength of the fluorescence signal (viability) can be noticed.The cells with seeding cell number of 1'000 cells/cm^2 didn't enter exponential growth at all while the cells with the highest seeding density reached 100% confluency already after 3 days. The evenly numbered pictures demonstrate the decrease of glucose concentration. Again, these data are inline with the information obtained from the viability assay as well as the microscopic analysis. Picture 4 shows no or little glucose consumption over the first 3 days after cell seeding whereas picture 8 reflecting the group with the highest seeding density points to a short lag followed by an exponential phase. No difference in cell morphology between flasks without sensors and those with sensors was seen (data on request). A clear correlation between the initial seeding density, the degree of confuency after 3 d and the glucose pattern can be seen. In addition, viability of the cells reaching confluency at day 3 started to stagnate and decrease over the period of day 4-6.



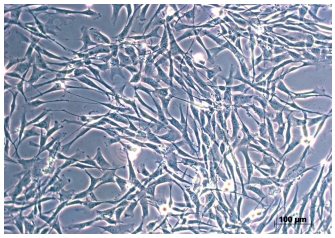
Picture 9: Viability (PrestoBlue, fluorescence units were measured) of the three different starting cell numbers after days 1 – 6 (1'000, 5'000, and 20'000 cells / cm²).



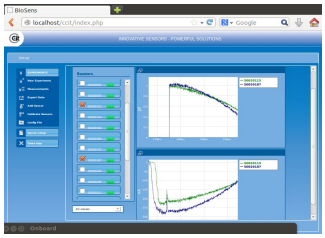
Picture 3: Inoculum 1'000 cells / cm^2



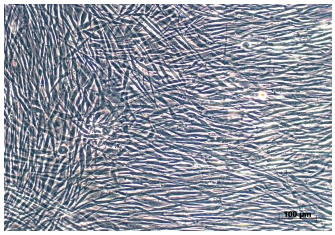
Picture 4: Inoculum 1'000 cells / cm^2



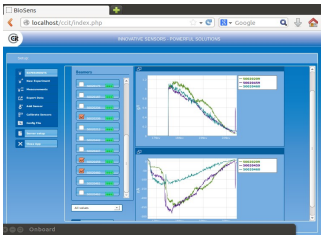
Picture 5: Inoculum 5'000 cells / cm^2



Picture 6: Inoculum 5'000 cells / cm^2



Picture 7: Inoculum 20'000 cells / cm^2



Picture 8: Inoculum 20'000 cells / cm^2

Discussion and Outlook

The results obtained demonstrate the feasibility of using CITSens Bio as an online cell culture monitoring tool, applicable over a period of at least 6 d and sensitive enough to detect minor changes between 0 and 1 g/l glucose. The use of CITSens Bio allowed to leave the cell cultures undisturbed within a closed incubator under stable carbondioxid and temperature conditions. **No need for visual checks or sample taking**. CITSens Bio not only zooms in on a random spot of your growing tissue surface but it delivers precise data on the immediate metabolic status of the complete culture. Numerous findings suggest that frequent opening of incubator doors negatively affects the growth and behaviour of cells (*Enhancing Data Quality with a Partly Controllable System at Shake Flask Scale. C. Klinger et al., BioProcess International 10(9), October 2012, 68-72*). CITSens Bio addresses this problem with a direct, simple, and cost-effective approach of what might be called «**smart cell culture monitoring**»: analyze the cells' **total environment, in-situ** and deliver **realtime** information on what is going on instead of measuring processed samples offline in distance to the point of sampling. **CITSens Bio is your cells' «eyes and ears»** allowing you to determine the right time for intervening with your culture, be it for harvest, split, media change, induction of differentiation and more.

Future work will focus on CITSens Bio's further development into a toxicity assay system (multiwell plates with integrated sensors) in order to monitor changes in glucose oxidation based on compounds added to given growing cell cultures.

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