APPLICATION NOTE

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Determination of cell proliferation using the CELLCYTE X™ live-cell imaging system

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Abstract

Live-cell imaging combined with an analysis software is a powerful tool that can provide researchers with direct quantitative data on cell proliferation, using acquired phase contrast and fluorescent images analyzed with detection software. In this application note, we explore the use of live-cell imaging to determine proliferation of label-free cells. The CELLCYTE X™ live-cell imaging system was used to acquire images of A549 (lung carcinoma), MCF-7 (breast cancer) and HepG2 (hepatocellular carcinoma) cells in the Enhanced Contour (EC) channel, and the detection software determined cell proliferation by measuring cell confluence. The results support the use of live-cell imaging to measure cell proliferation in label-free cells, using cell confluence.

Introduction

Cell proliferation, a widely used parameter to evaluate the capacity of cells to grow and reproduce, reflects the dynamic process between cell division and cell death in a population. The significance of cell proliferation lies in its ability to indicate the status of a cell culture—for example, it can reveal the cytotoxic or cytostatic effect of a drug treatment (Aysun, 2016).

There are a variety of methods to determine cell proliferation through indirect and direct measurements, which can be performed as time-course or end-point experiments. Conventional techniques, like manual cell count using permeable viability dyes, are useful but present disadvantages because the user's experience plays an important role in the results, and the sample is usually damaged during the readings, making it impossible to use the same cell population over time. Additionally, some cell proliferation techniques cannot be modified to perform high-throughput experiments, therefore they become very time-consuming when handling a large number of samples. To overcome these obstacles, researchers have developed techniques to directly measure cell proliferation using microscopy (Drey, 2013; Jaccard, 2014). Among the parameters used are cell confluence, cell count and nuclei count.

Cell confluence measures the area covered by cells in a given field of view observed under an inverted microscope. This parameter has been used for several years while working with adherent cell cultures and is a useful way to determine when cells are ready to perform an experiment or need to be split, as well as to evaluate growth, behavior and health. Typically, cell confluence has been performed by estimating a subjective value, but this is a non-ideal and inaccurate method because individuals estimate based on their own perceptions. Nevertheless, the advancement of computer programs has made it possible for researchers to develop methods to obtain accurate cell confluence measurements (Busschots, 2015), eliminating the varying perceptions of individual users. The more precise the detection of the program is, the more accurate the results will be. Nowadays, cell confluence determined by software applications is an accepted direct method to monitor cell proliferation in adherent cells since an increase in cell number correlates with an increase in cell confluence. In this application note, we show how our CELLCYTE X™ system can determine cell proliferation by measuring cell confluence in a label free manner with the system's Enhanced Contour (EC) channel.

Material and methods

Cell lines and cell culture conditions

A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic. MCF-7 cells were cultured using EMEM supplemented with 0.01 mg/mL human recombinant insulin and 10% FBS. HepG2 cells were cultured in Minimum Essential Medium supplemented with 10% FBS, 1% GlutaMAX, 1% antibiotic/antimycotic, 1% non-essential amino acids and 1% sodium pyruvate. All the cell lines were cultured at 37°C, 5% CO_2 and <95% humidity.

Quality control of cell cultures

Per well, 3x103 A549, 15x103 MCF-7 and 10x103 HepG2 cells were seeded into 96-well plates. The cells were monitored for 96 hours, two images per well were acquired every 3 hours using the 10X objective of the CELLCYTE X™ live-cell imaging system. A total of 33 scans were performed. The images were analyzed using the CELLCYTE™ Analysis software to create an accurate detection mask for cell confluence by using a cell proliferation recipe for each of the cell lines. The experiments were performed in triplicate.

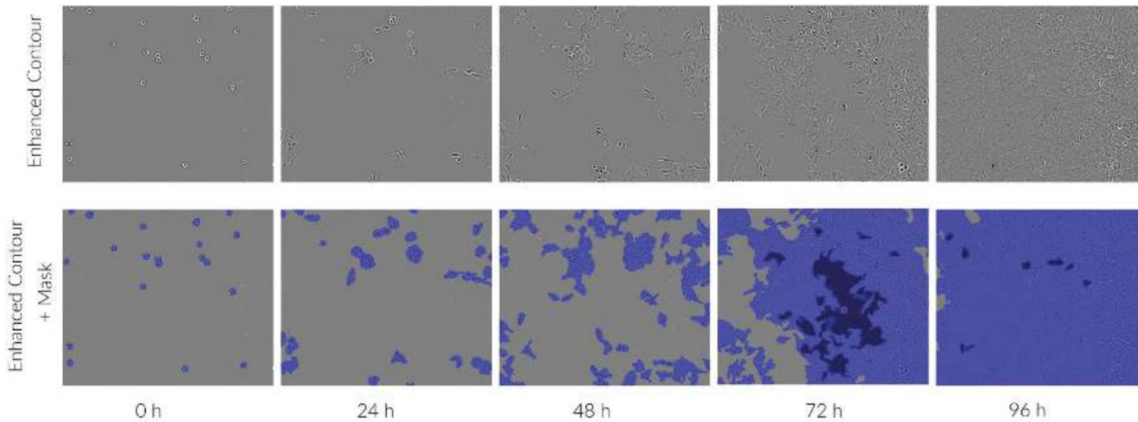

Figure 1: *A549 cell proliferation detection mask representation. The EC images + mask images show the cell confluence detection mask created by using a recipe for cell proliferation.*

Results and discussion

Monitor cell proliferation for quality control of cell cultures

Live-cell imaging has emerged as a helpful technique in cell culture, and together with accurate software, it can provide precise quantitative data. One common application for this technique is to aid in the routine work in cell culture by serving as a quality control that includes detailed information about the growth curve, morphology and growing patterns of the cultures. In this application note, we performed cell proliferation experiments using EC images of three different cell lines (A549, MCF-7 and HepG2). **Figure 1** shows EC images with and without their detection mask created by using a recipe for cell proliferation, the images are examples of A549 cell proliferation over time. **Figure 2** shows the cell confluence graphs for A549, MCF-7 and HepG2 cells. The proliferation curves follow the cells from low to high confluence over a period of 96 hours. Each cell line presents its own growth curve, reflecting the nature of its growing patterns (Giard, 1979; Aden, 1979). A549 cells grow in monolayers that spread uniformly, while MCF-7 and HepG2 cells aggregate to form islands. A549 cells have a faster doubling time compared to MCF-7 and HepG2 cells, explaining why they reached high confluence even if the initial confluence was lower than for the other two cell lines. Our results demonstrate EC images of label-free cells acquired and analyzed using the CELLCYTE X™ system are a useful tool to monitor cell proliferation and the general health of the cell cultures by detecting cell morphology and growing characteristics

under different experimental environments. The growth curves obtained using label-free cells are a good indication of the behavior of the cell culture. Well-reported parameters such as doubling time can be compared and used to determine if cells are changing over passages, helping to maintain good practice in cell culture as well as to detect discrepancies before performing experiments.

Conclusions and future direction

Cell culture is a crucial pillar in different research fields, and consistency and reliable data are basic requisites to guarantee reliable results. Routine quality control of cell cultures should be performed to ensure the cells being cultured behave as expected, but these quality controls should not disturb or destroy the samples. Taking this into account, live-cell imaging of label-free cells emerges as an ideal technique to accomplish this task. In this application note, EC live-cell images acquired by the CELLCYTE X™ system were analyzed to measure cell proliferation over time; as a quality control for general health of the culture, providing evidence of how label-free cells can be used to determine cell proliferation using cell confluence.

Altogether, the present application note demonstrates that live-cell imaging using the CELLCYTE X™ system is able to detect cells with different morphologies and growth patterns in EC channel under diverse experimental conditions, providing accurate quantitative measurements of cell proliferation.

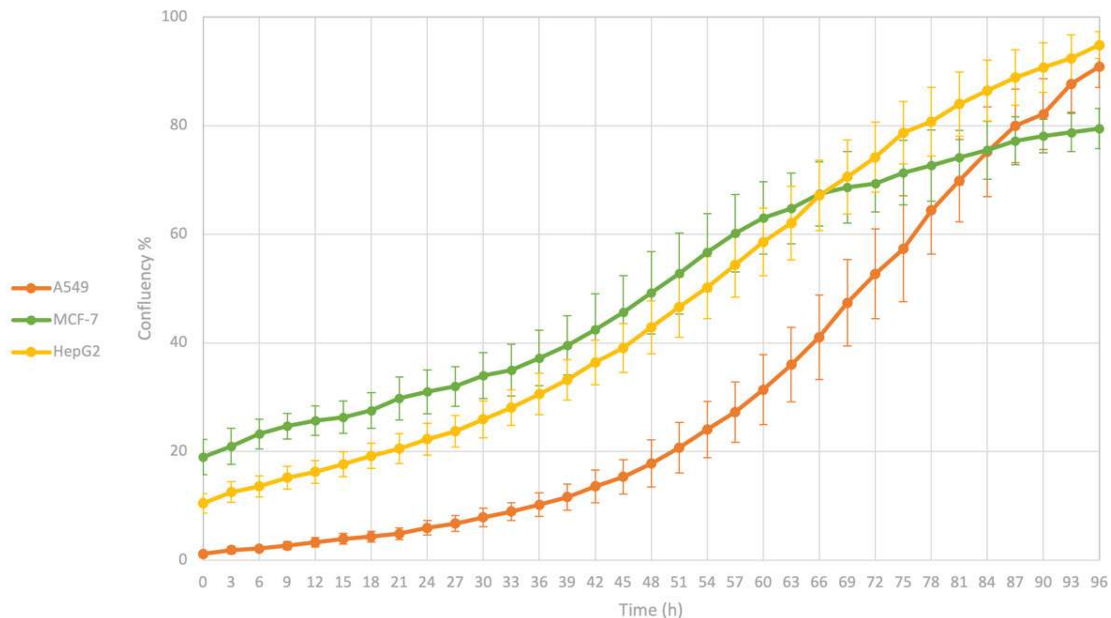

Figure 2: *Confluence graph following A549, MCF-7 and HepG2 cell proliferation. The cell lines were seeded at low cell confluence and monitored over time using 10X objective in the CELLCYTE X's™ EC channel.*

References

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