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Evaluation of the "Cellscreen" system for proliferation studies on liver progenitor cells

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Abstract

Proliferation studies on mammalian cells have been disadvantaged by the limited availability of non-invasive assays as the majority of approaches are based on chemical treatment, sampling or staining of cells removed from culture. In this study, we utilised the Cellscreen system (Innovatis AG, Bielefeld, Germany), a non-invasive automated technique for measuring proliferation of adherent and suspension cells over time. We have evaluated the ability of the Cellscreen system to monitor and quantify growth of adherent liver progenitor cells over time and tested several applications, (i) serum reduction or (ii) treatment with a cytokine. Our results demonstrate that the Cellscreen system reproducibly documents pro- and anti-proliferative effects of cytokines and growth factors and quantifies changes by providing cell-doubling times for control and test cultures. However, we found that for the conversion of cell density values into absolute cell numbers different conversion factors, which better suit the individual growth phases, need to be established. Collectively, these findings reveal that the Cellscreen system is applicable for the determination of cell proliferation of adherent and suspension cells in response to a variety of (growth) factors. It minimises operator participation and thus enables more rapid and larger screens and, being non-invasive, permits multiple assays on the same culture of cells. Hence, this technique proves superior to the common proliferation assays opening up new dimensions of proliferation studies in cell biology.

Keywords: Proliferation; Cell growth; Non-invasive measurement; Adherent cells

Introduction

In vitro experiments involving mammalian cell culture are crucial for identification and characterisation of factors responsible for their proliferation and differentiation. We wish to identify factors that are essential

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for proliferation of liver progenitor cells (LPC), which are often referred to as oval cells in rodent models because of the ovoid shape of their nucleus (Farber, 1956). As these cells are at least bipotential, i.e. they can differentiate into hepatocytes as well as bile duct cells, they are considered good candidates for cell therapy to treat chronic liver disease. Factors which enhance their proliferation can be used to expand their numbers both in vivo and in vitro. To study these cells in more detail we have generated two LPC lines, one from embryonic and the other from adult mice. These are termed bipotential murine embryonic liver (BMEL) cells and bipotential murine oval liver (BMOL) cells, respectively. Some signalling pathways and factors involved in proliferation of LPCs have been elucidated (Knight et al., 2005; Lowes et al., 2003; Matthews and Yeoh, 2005).

However, analysing the effects of agents on cell proliferation has been limited by assays available to measure cell growth and proliferation. These assays are invasive as it is necessary to treat cells with chemicals such as 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) which kill them in the process. The need to sacrifice cells at each time point mandates a comparison between replicate samples harvested at progressive stages to obtain a time course which may not be valid in some experimental conditions. Furthermore, for statistical significance a large number of replicate samples for each condition may be required implying a lot of material and time to process.

To overcome this problem we looked at the Cellscreen system (CS) (Innovatis AG, Bielefeld, Germany). It provides a novel non-invasive method which continuously measures cell growth of the same culture of cells. As the measurements are performed in situ it is possible to follow the same cells or clones and to perform additional assays at the end of the measurement period leaving valuable cells unaltered and intact. Suspension or adherent cells are cultured in microtitre plates and measured for growth using the proliferation of suspension cells (PS) mode (Brinkmann et al., 2002) or the proliferation of adherent cells (PA) mode, respectively. This approach allows continuous monitoring of growth instead of selecting individual time points. The use of microtitre plates for culturing facilitates inclusion of large sets of samples.

The CS consists of a microscopy unit that collects digital images of the cells which are cultured in microtitre plates. The connected computer is equipped with special pattern recognition software which analyses the cell density of either suspension or adherent cells using different modules. As all images and analyses are archived on a hard drive it is possible to ascertain the morphology of cells retrospectively to validate the results and even to re-process the data with different parameters.

The aim of this study was to evaluate the performance of the CS as an advanced approach to measure growth of our adherent LPC lines. Besides, we wished to establish a correlation between cell density of adherent cells and cell number for future assays. In the present study we show that proliferation of LPCs can be monitored continuously using the CS without affecting cell cultures. However, a conversion from density values generated by the instrument into cell numbers was found to be unreliable for some growth phases of LPC cultures because the morphology of the cells changed with progressing time of culture. The performance of the CS in measuring proliferation was evaluated using two different applications: (1) reduction of foetal bovine serum (FBS) or (2) treatment with tumour necrosis factor alpha (TNFα). The system detected reliably a decrease or increase in cell proliferation, respectively.

Materials and methods

Cell culture

LPC lines were cultivated in T25 tissue culture flasks (Nunc, Rochester NY, USA) in Williams' E medium (Invitrogen, Mulgrave VIC, Australia) plus additives: insulin-like growth factor II: 30 ng/ml (GroPep, Adelaide SA, Australia); epidermal growth factor: 20 ng/ml BD Biosciences, North Ryde NSW); humulin: 10 μg/ml (Eli Lilly Australia, West Ryde NSW, Australia); fungizone: 2.5 μg/ml (Gibco, Mulgrave VIC, Australia); penicillin G: 44.8 ng/ml (Behring Diagnostics, La Jolla CA, USA); streptomycin: 675 ng/ml (Gibco); glutamine: 200 nM (Sigma-Aldrich, Castle Hill NSW, Australia); foetal bovine serum (FBS): 0–10% (Invitrogen) depending on the cell type and experimental conditions at 37 °C, 5% CO_2. For experiments, the cells were seeded in 24- or 96-well microtitre plates (BD Biosciences or Nalge Nunc International, Rochester, NY, USA) in 1 ml or 200 μl of medium, respectively.

The Cellscreen system

The CS consists of an inverted phase microscope (IX 50, Olympus) equipped with 4 × and 10 × objectives to measure growth of adherent and suspension cells, respectively; a motorised X–Y stage and a CCD camera (KPF100, Hitachi) with a resolution of 1024 × 1024 pixels. Special pattern recognition software, an adapted form of CeDeX image recognition (Guderman et al., 1997), analyses captured images. The settings for the experiment, the acquisition as well as the results are stored in a database on a linked personal computer which can be accessed through the graphical user interface.