Novel Plate-Based Cell Viability Assay System

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Abstract
To develop a novel, homogenous, fluorescence-based assay system for detecting cell viability and test its applicability to proliferation assays and cytotoxicity testing of pharmacological compounds.

Introduction
Traditional methods of detecting cell growth or death require the addition of agents that are radioactive or that perturb the biological system of the cell.
BD Biosciences Discovery Labware has developed a user-friendly assay format that is suitable to monitor the growth or death of a wide variety of cells, including mammalian, insect, bacterial and yeast cells. The assay is non-destructive to cells and allows multiple reading for kinetic analysis and does not require the addition of any reagents. This system uses a sensor that is physically removed from the cells so that the cells may be further processed for other assays and bio-chemical character-izations. We present results of growth, proliferation and cytotoxicity assays using the BD™ Oxygen Biosensor System format.

Methods
Using several cell types, including mammalian leukocyte and epithelial cells and insect cells, we investigated the dependence of the fluorescent signal on cell density. Increasing fluorescence represents a decrease in oxygen concentration, which may be correlated to an increase cell number.

Results
For all cell types tested, the fluorescent signal of our biosensor was proportional to cell number over a wide range of cell densities. Increased cell number resulting from either higher seeding density or proliferation of cells in response to growth stimulus gave increased fluorescent signal.

Conclusions
This cell viability system, based on a simple-to-use fluorescence biosensor technology, can be used for quantitative proliferation testing of pharmacological compounds. The assay platform does not interfere with cell growth and provides an inert tissue culture environment. We expect that this easy-to-use, automation-friendly system will have multiple applications in drug discovery and development.

Quantitate Growth and Proliferation

(A) (B) (C)

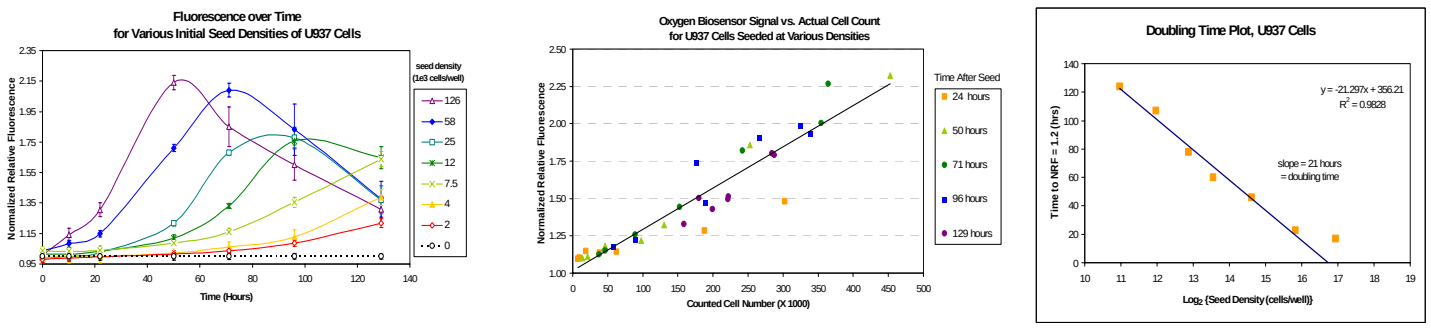

Figure 1. **(A)** U937 cells were added at varying densities across the plate and monitored over time on a standard fluorometer. Larger seed densities gave rise to signal sooner, and in all cases signal increased over time as the cells increased in number. Such nested growth curves have been observed for a very large number of cell types, including mammalian and insect cell lines, bacteria, and fungi. A simple proliferation experiment of this sort allows the user to decide which number of cells is appropriate, depending upon the time duration of the assay (see ***Figure 2***). **(B)** Cells from parallel wells were physically counted with a hemocytometer. It can be seen that cell number correlated closely with BD OBS fluorescence, regardless of the initial seed density or amount of time in culture. **(C)** A plot of the time required for each seed density to reach a given threshold value of NRFU as a function of seed density allows one to calculate the actual doubling time of the cells. This approach can be used to understand the impact of media composition on proliferation, which information could be used to optimize culture conditions or understand the impact upon proliferation of a drug.

Compatible With a Wide Variety of Cell Types

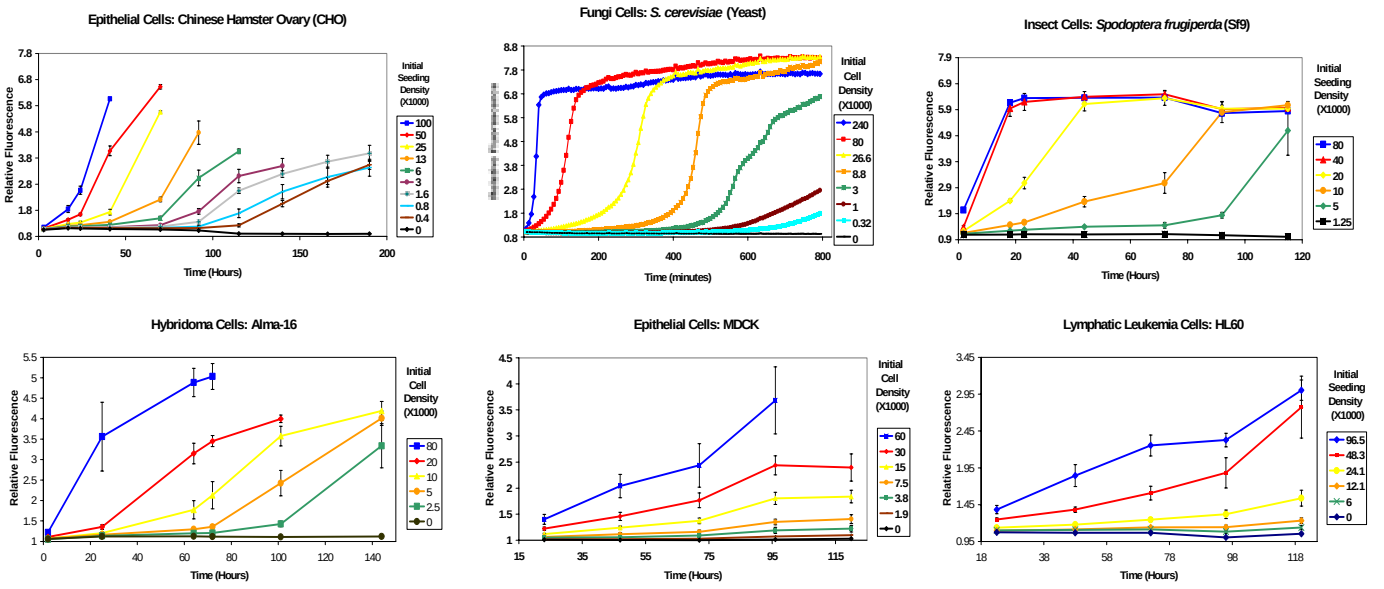

Figure 2. The BD Oxygen Biosensor System supports a wide variety of cell types. Growth experiments akin to that described in in Figure 1a were conducted for a variety of cell types. Fluorescence was monitored over time with a standard fluorometer (BMG Polarstar). The maximum increase in signal varied from 2- to 8-fold above background, depending on the cell type used. The maximum fluorescence that each cell type can achieve indicates the lowest oxygen concentration where proliferation under the given conditions can be maintained. Data are plotted as the mean of 5 replicates with the the standard deviation as the error bar.

Observe / Optimize Culture of Anchorage-Dependent Cells on Supports

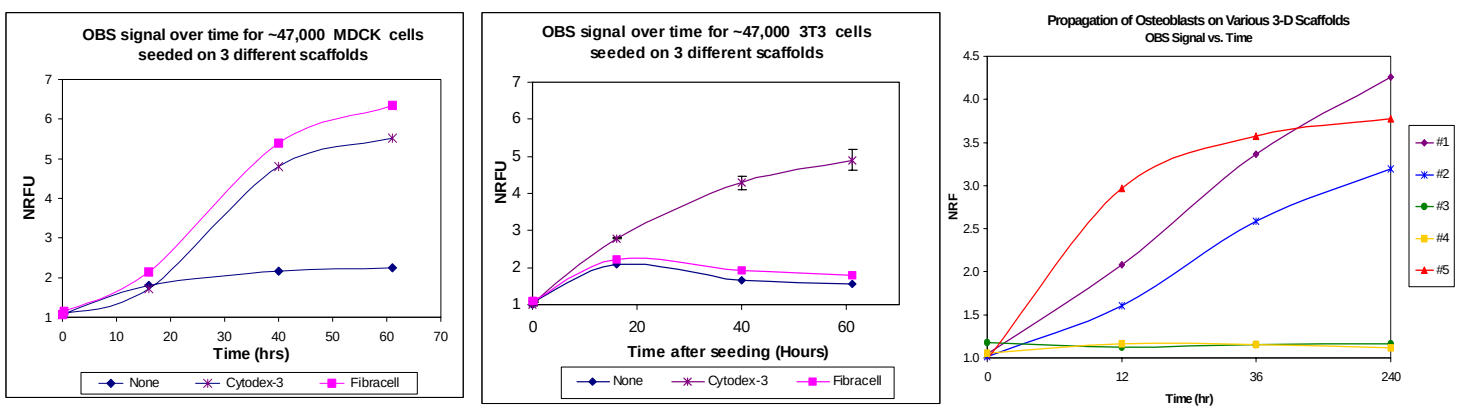

Figure 3. **(A)** MDCK cells will proliferate on BD OBS in the absence of a support, but under these particular growth conditions fared much better on either Cytodex-3 (collagen-coated) or Fibracell (naked) bioreactor supports. **(B)** 3T3's cannot survive more than 24 hours on BD OBS without an ECM-coated support, but do nicely on Cytodex-3. **(C)** Osteoblast proliferation on various 3-D scaffolds indicate varying abilities of such substrates to support proliferation; scaffolds #1, 2, and 5 clearly support proliferation, whereas #3 and #4 do not.

Readily Observe Stimulation or Suppression of Normal Cell Growth

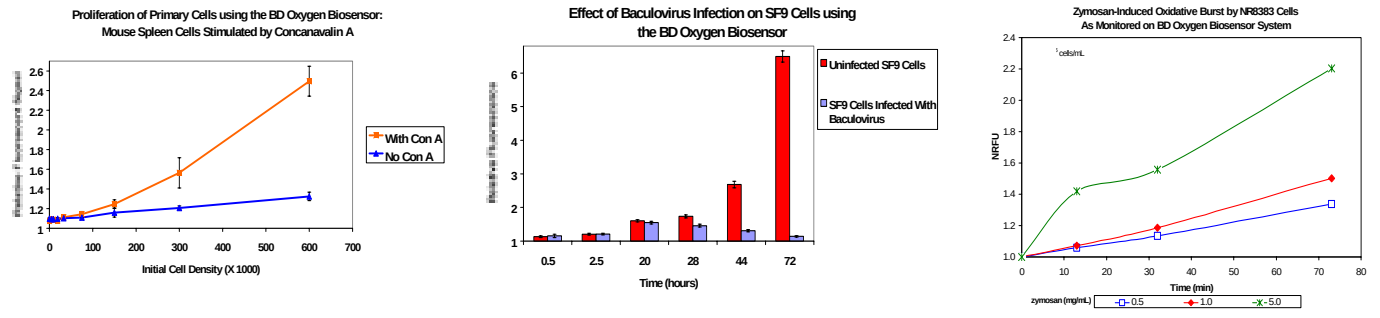

Figure 3. Proliferation of cells can be easily measured. **(A)** Mouse spleen cells were isolated and stimulated with a known proliferative agent, concanavalin A. Marked stimulation was evident after 17 hours on the BD Oxygen Biosensor System. Cell counts verified that the wells receiving the treatment had significantly more cells than non-stimulated cells (data not shown). Data are plotted as the mean of 3 replicates with the error as the standard deviation. **(B)** SF9 insect cells were infected with baculovirus and monitored over 3 days. After 44 hours, infected cells are enlarged and growth is arrested. After 72 hours, the cells lyse. The infective process can be easily monitored with the BD OBS. Data are plotted as the mean of 3 replicates with the error as the standard deviation. **(C)** NR8383 cells were challenged with increasing concentrations of zymosan, which clearly induced an increase in oxygen consumption. Measurement of superoxide production by cytochrome C (not shown) correlates to the increase in oxygen consumption..