

Upgrading Cell-Based Viability Assays

Novel Approaches More Accurately Reflect the In Vivo Human State

K. John Morrow Jr., Ph.D.

Pharmas and biotechs struggle to screen large quantities of compounds, hoping to eliminate early those that would prove more destructive than beneficial. One avenue toward a successful resolution of this challenge is the cell-based viability assay, a rapid and convenient technique for use in drug discovery. A number of companies have taken advantage of novel approaches to offer a variety of innovative upgrades to this platform.

Animal testing of toxic compounds was widely used in the past as an alternative to classic in vitro testing methods of viability and cytotoxicity, such as dye-exclusion or colony-formation assays. These were laborious approaches—time-consuming, insensitive, and poorly suited for high-throughput screening. But animal testing is expensive and also low throughput. Moreover, ethical concerns over the infliction of severe pain and suffering to sentient creatures, combined with the inadequacy of animal models due to species differences, have motivated the development of cell-based assays that truly reflect the in vivo human state.

Genotoxicity Assays

Gentronix (www.gentronix.co.uk) was founded in 1999 by Richard Walmsley, Ph.D., with a yeast cell-based genotoxicity assay as the original technology. Today, the company seeks to minimize late preclinical failure by eliminating unsatisfactory candidates before they advance to these later stages of evaluation.

According to Dr. Walmsley, the ICH guidelines mandate that regulatory genotoxicity assessment be performed before the candidate enters into clinical trials. Positive genotoxicity data can lead to costly delays in clinical trials while further in vitro and in vivo studies are carried out to make a full risk assessment. That is why screening much earlier than preclinical safety assessment is a good idea.

To meet these challenges, the company has developed the mammalian cell GreenScreen HC platform, named after its use of the green fluorescent protein reporter. The reporter responds to the GADD45a gene, which mediates the adaptive response to genotoxic stress. Its complex regulatory elements respond to all classes of genotox-

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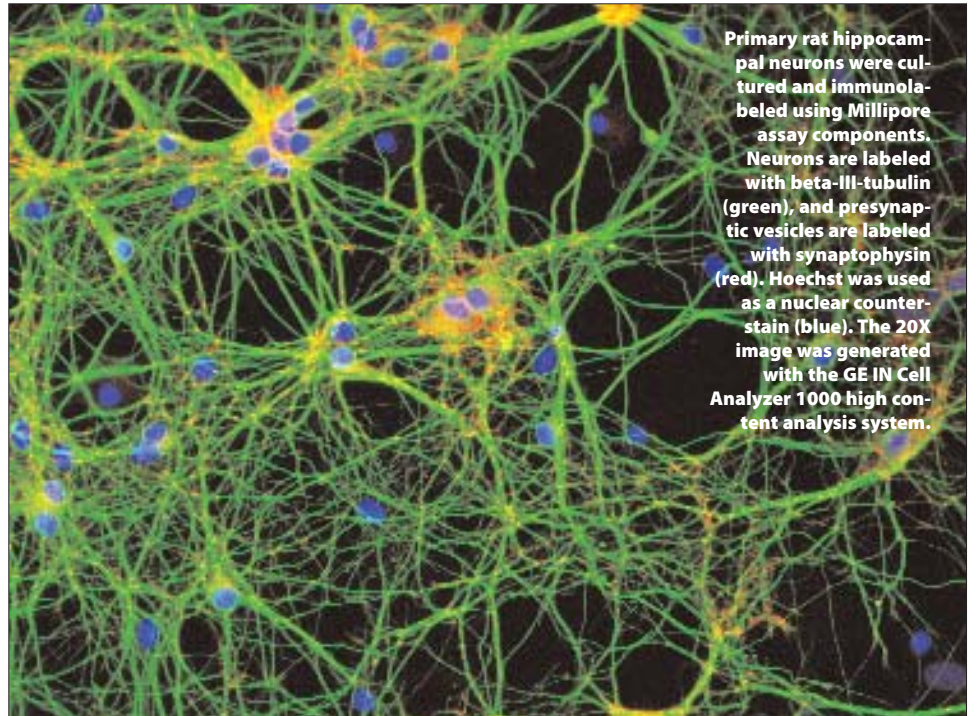
ins including direct-acting mutagens, clastogens, and aneugens resulting in a fluorescence reaction in responsive cells.

The procedure is extremely rapid—yielding results over a two-day period—and has been validated in a number of studies published in peer-reviewed journals. The goal is to help narrow large numbers of potential compounds down to the handful that will be viable drug candidates. An important feature of the platform is its ability to detect promutagens and procarcinogens—compounds that must be converted by liver enzymes to an active form from their initial inactive state.

Solutions

“We approach cell-based assays on two fundamental levels—providing solutions for characterizing cellular events across a wide range of cell types and using cells as biological test tubes to engineer specific assays for research and drug discovery,” says Jeff Till, Ph.D., marketing director for drug discovery at **Millipore** (www.millipore.com). The company has introduced kits for studying cellular toxicity using high-content image analysis.

“We cover a range of cellular toxicity responses such as DNA damage and oxidative stress,” Dr. Till says. A number of new



Primary rat hippocampal neurons were cultured and immunolabeled using Millipore assay components. Neurons are labeled with beta-III-tubulin (green), and presynaptic vesicles are labeled with synaptophysin (red). Hoechst was used as a nuclear counterstain (blue). The 20X image was generated with the GE IN Cell Analyzer 1000 high content analysis system.

products focus on assessment of the effects of drugs on the nervous system. These include a screening kit composed of high-quality, validated, target-specific detection reagents for profiling neurotoxicity, neurite outgrowth, and neuronal morphology in a wide variety of mammalian cell types.

Additional cell-based assay products available from Millipore include cell-signaling kits designed around a variety of cell lines engineered for drug discovery through the use of specific reporter systems.

Noteworthy are mouse and human stem cell lines for the investigation of compounds that affect cellular differentiation.

Gene Manipulation in Living Cells

Sigma-Aldrich (www.sigmaaldrich.com) and **Sangamo Biosciences** (www.sangamo.com) have partnered to exploit an interventional technology in genome science for the development of new material for cell-based assays.

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News Discovery & Development Briefs

NBC Pockets \$46M from NIAID

The Northeast Biodefense Center (NBC) received a \$46 million NIAID grant that will allow the continuation of research activities in emerging infectious diseases. NBC comprises 350 scientists and 28 institutions in New York, New Jersey, and Connecticut. Established in 2002, it is reportedly the largest of NIH's 11 Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases in the nation.

Roche To Pay \$4.25M to Use Halozyne Technology

Roche (www.roche.com) has selected a fifth biologic target under an existing license and collaboration agreement with Halozyne Therapeutics (www.halozyne.com) and will pay \$4.25 million for exclusive, global rights to use Halozyne's recombinant human hyaluronidase, rHuPH20, with this target.

In December 2006, Roche paid Halozyne \$20 million up front to apply the Enhance™ Technology, a drug delivery

platform, to three biological therapeutic compounds. The deal also allowed it to select up to 10 additional targets.

All told, the deal has a value of over \$601 million plus royalties: \$20 million up front, \$111 million in clinical, regulatory, and sales milestones related to the first three targets, and up to \$47 million in initial and milestone fees for each of the additional 10 targets.

AstraZeneca and U.K. Institutes to Target Molecular Chaperones

AstraZeneca (www.astrazeneca.com), Cancer Research Technology (www.cancertechnology.co.uk), and the Institute of Cancer Research (ICR) are joining forces to discover and develop new drugs targeting molecular chaperones that support cancer cell growth. AstraZeneca will invest £4 million (almost \$6.4 million) over the next three years, and Cancer Research U.K. will provide another £1.6 million (\$2.55 million) to start research at ICR.

The work will focus on the search for chaperone pathway proteins excluding

HSP90 that could be targeted to block the growth of cancer cells. Under terms of the agreement, AstraZeneca retains an exclusive, worldwide license to commercialize compounds developed in the collaboration.

Genentech Pays Bayhill \$25M for Diabetes Therapy

Genentech (www.gene.com) is paying Bayhill Therapeutics (www.bayhilltx.com) \$25 million up front in cash and equity for rights to its type 1 diabetes treatment. BHT-3021 is currently in a Phase I/II trial. If successfully developed, approved, and commercialized, Bayhill could receive over \$325 million.

Under the terms of the exclusive, worldwide license, Bayhill will be responsible for completing the ongoing clinical studies, and Genentech will reimburse related expenses. Genentech will then be fully responsible for all future R&D, manufacturing, and commercialization activities.

Bayhill will also receive escalating royalties on annual net sales. The firm also retains rights to opt-in on future development and

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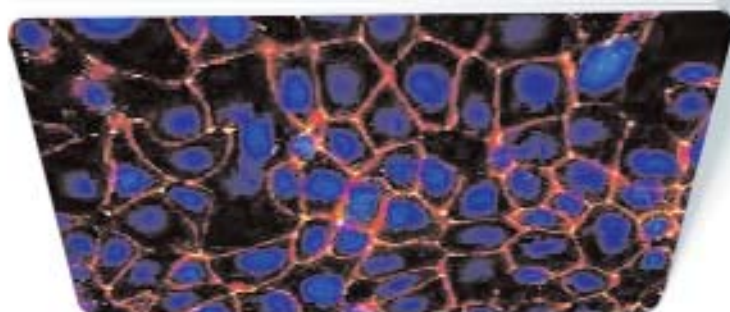
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Cell Viability Assays

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“Using a zinc finger nuclease-based approach we are now able to engineer the genome of living cells,” states David Smoller, Ph.D., president of the research biotechnology business unit at Sigma-Aldrich.

The CompoZr™ zinc finger nuclease (ZFN) technology for targeted genome editing allows the insertion, deletion, or alteration of targeted sequences, including the placement of a reporter gene in front or at the 3' end of any endogenous locus that the investigator may choose.

The technology is modular—the zinc finger domain targets to a specific sequence and the heterodimeric nuclease cleaves the genomic DNA, creating a double-stranded break. This break in the DNA stimulates the natural repair mechanisms of the cell, allowing an exogenous transfected DNA sequence to be integrated into the region of interest in the genome.

The CompoZr ZFN system allows the investigator to engineer a wide range of possibilities for custom cell lines to be used in cell-based assays. The procedure is fast and straightforward, and because it can be performed on living cells, it would lend itself especially to redesigning embryos, including *Drosophila*, zebrafish, mice, and other important molecular model systems.

Molecular Probes

Invitrogen (www.invitrogen.com), a division of Life Technologies, has available through its Molecular Probes brand a wide variety of options for assaying cellular viability and cytotoxicity. These encompass a number of fluorometric assays that possess significant advantages over traditional colorimetric and radioactivity-based approaches.

Mike Janes, manager of R&D for Invitrogen's cell health assays business, and George Hanson, Ph.D., principal scientist for discovery assays and services, elaborate on the company's products, comparing automated high-content cellular imaging with high-throughput screening technologies.

The two strategies are appropriate for different types of investigations, since cellular imaging allows an overall look at a mixed population of cells, but lacks the speed of high-throughput screening. While some Invitrogen products employ classical approaches to measure general cell viability and cytotoxicity, the Click-iT® platform is the basis upon which new assays for apoptosis and nascent synthesis of DNA, RNA, or protein have been developed.

According to Janes, Invitrogen's development of capabilities to investigate perturbations of lipid metabolism is a recent new direction for the company. The HCS LipidTOX™ kits are designed for the detection of phospholipidosis and steatosis; they offer a complete solution for performing image-based high-content screening assays. The toxic side effects of disrupted lipid metabolism can be triggered by various foreign compounds. The probes are available in a range of colors, detectable with fluorescent microscopes and quantifi-

able with image analysis software available on HCS readers.

Multiplexed Assays

Promega (www.promega.com) offers a number of cell-based assays that can be combined in the same well, based on antibody or fluorescence imaging systems. Moreover, the newer assay chemistries are easy to implement on robotics platforms and scalable up to 1,536-well plates,

within a single assay.

Another key product is the nonlytic, luminescent CytoTox-Glo™ cytotoxicity assay. “It can detect small changes in viable cell number and also can be multiplexed with fluorescent-based assays, improving data quality and saving time, money, and reagents,” says Guthmiller.

Stem Cell Isolation Technology

With stem cell technology in the public

tent stem cell sorting and analysis kit.

Intelligent Cells Wired for Assays

Roche Diagnostics' (www.roche.com) xCELLigence System has a microelectronic biosensor built into each well of a standard 96-well microtiter plate, says Burkhard Ziebolz, Ph.D., head of global communication. This allows any change in cell number, cell morphology, or cell attachment to be detected in real-time, without the need for labeling or reporters.

Roche has introduced instrumentation for reading the plates, the first of which was the xCELLigence RTCA SP device followed by a newer upgrade, the xCELLigence RTCA MP Instrument. The MP Instrument has a higher throughput capacity and is more versatile, featuring six E-Plate 96's as opposed to only one in the RTCA SP. Since each E-Plate can be individually manipulated, a number of researchers can work simultaneously, making the device useful for pharmaceutical investigations.

Monitoring Cellular Performance

“Mitochondria were long neglected as a tool for cell-based assays,” says Steve Chomicz, vp of sales and marketing at Seahorse Bioscience (www.seahorsebio.com). Although their central roles in cancer and many other pathological processes have been long recognized, the tools for studying mitochondrial function have hardly changed since the 1930s.

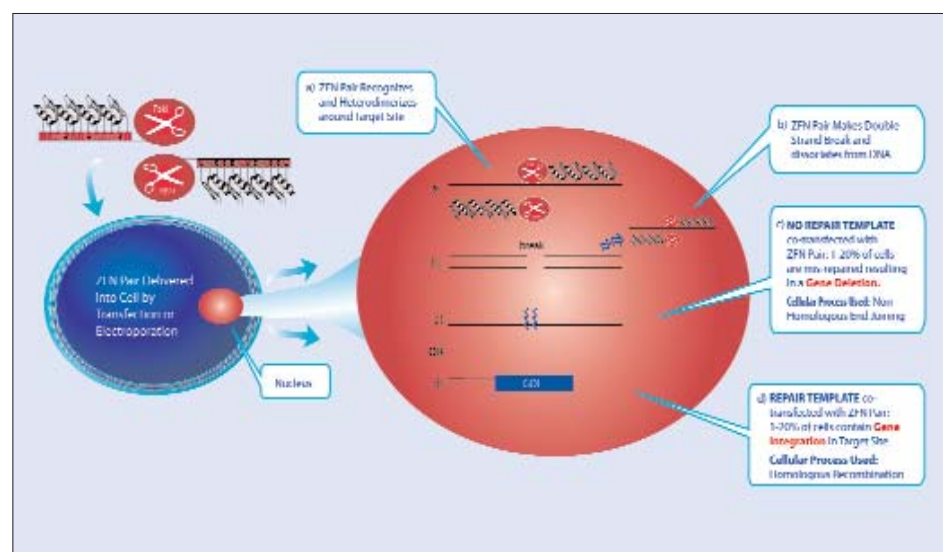
According to Chomicz, many cell-based assays measure features such as cell permeability, and in doing so miss the vital contribution of mitochondrial health to cell function. “We perform the equivalent of a stress test on our cells that reveals dysfunctional respiratory capacity,” he explains.

It is now possible to monitor myocytes grown under conditions that mimic a lean or obese model in order to develop a realistic cell-based assay for studying drugs that may affect metabolism. This includes the measurement of cellular respiration, revealing important cellular responses not detectable with other viability assays. An important feature of the XF platform is its noninvasive, physiologic measurement for primary or cultured cells, allowing the cells to be recycled for other purposes.

The Future of Cell-Based Assays

According to Andrew Niles, Ph.D., and his colleagues at Promega, there is a tendency within the industry to move to bioluminescent assays and away from traditional absorbance and fluorescence chemistries.

Bioluminescent proteins have a number of advantages over conventional organic fluorescent dyes. They are highly restricted within the kingdom of living organisms, are much more sensitive, and are rarely subject to quenching or interference through autofluorescence. However, new ways of examining cellular response are on the horizon, and being label-free is especially tantalizing.



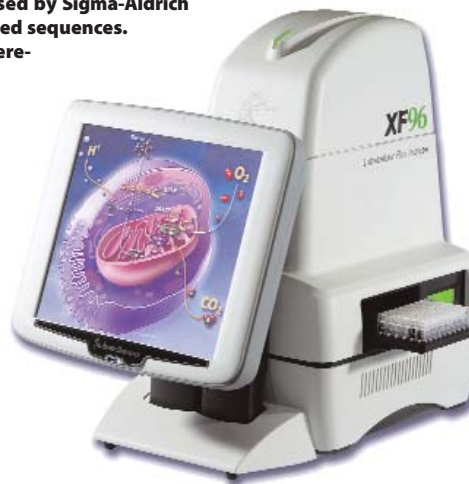
The zinc finger nuclease (ZFN)-based approach used by Sigma-Aldrich allows the insertion, deletion, or alteration of targeted sequences. The procedure can be performed on living cells, therefore lending itself to redesigning embryos of *Drosophila*, zebrafish, or mice, according to the company.

according to Pam Guthmiller, strategic marketing manager for cellular analysis.

“By developing assay chemistries that can be multiplexed in the same well, the researcher can now perform these assays in microwell plates using a simple multi-mode detection instrument. Because of the simplicity and sensitivity, a number of life science researchers are turning to these formats.”

The offerings are versatile and sensitive, according to Guthmiller, and include the GloMax®-Multi detection plate reader system for measuring a broad range of fluorescent and luminescent cell signals. Many of the assays are streamlined through a homogeneous add-mix-measure format, meaning that removing or changing the medium is unnecessary. The various substrates are simply added to the culture medium as a concentrate and then read. Examples include the MultiTox-Fluor multiplex cytotoxicity assay (measures viability and cytotoxicity) combined with the Caspase-Glo® 3/7 assay, designed to provide data on the role of apoptosis in cell cytotoxicity.

These assays can also be combined with reporter assays to gain information on gene expression when cells are exposed to potential therapeutic drugs. The ONE-Glo™ luciferase reporter assay system can measure transcription and translation. Combined with the CellTiter-Fluor™ cell viability assay, evidence can be assembled on the concordance between gene expression analysis, cell viability, and cytotoxicity



Seahorse Bioscience's XF96 Extracellular Flux Analyzer simultaneously measures the two major energy yielding pathways— aerobic respiration and glycolysis—in a convenient, microplate format.

eye, it should be noted that a number of products for stem cell research are on the marketplace. BD Biosciences (www.bdbiosciences.com), a division of Becton Dickinson, has a range of kits, individual reagents, flow cytometry and bioimaging instrumentation, and cell culture environments for advancing cell-based technologies.

BD Biosciences just introduced the BD™ mouse hematopoietic stem and progenitor cell (HSPC) isolation kit. According to Julia Lizondo, product manager of stem cell research reagents, the kit contains the reagents required for the flow cytometric isolation of hematopoietic progenitor and stem cells from mouse bone marrow samples.

The four-color kit consists of pretitrated fluorochrome conjugated antibodies, compensation beads, isotype controls, an Fc blocker, and a viability dye. In addition to the mouse HSPC isolation kit, BD Biosciences offers the BD™ human pluripo-