In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-007-9025-5

Animal Symposia

A-1

Global Diabetes Perspective and Diabetes R & D at Eli Lilly and Company. ANNE REIFEL MILLER. Research Fellow, Diabetes Drug Hunting Team, Eli Lilly and Company, Lilly Corporate Center, Bldg. 98C, Rm 2332, Indianapolis, IN 46285. Email: a.r.miller@lilly.com

The number of individuals with diabetes is increasing at an epidemic rate throughout the world. Diabetes affects more that 230 million people worldwide, and this number is expected to increase to 320 million by 2025. Each year, 7 million new cases of diabetes are identified. Over 3 millions deaths can be attributed directly to diabetes yearly, making diabetes the fourth leading cause of death by disease globally. In the United States alone, 20.8 million adults and children now have diabetes with 6.2 million of these cases undiagnosed, while another 41 million individuals have pre-diabetes. Current studies indicate that individuals with pre-diabetes have a 75% probability of developing diabetes within 30 years. By 2050, 12% of the US population is expected to have diabetes, accounting for a total of 48.3 million people. This number could be higher if the rate of obesity continues to rise among Americans. Diabetes is an expensive disease to treat. The US health care spending on diabetes and its complications reached $132 billion in 2002, with world wide spending, including direct and indirect costs, close to $286 billion. Analysts predict that the market for diabetes drugs is currently $10 billion and that this number could easily double. Eli Lilly and Company which introduced the first commercially available insulin in 1923 continues to identify and develop therapies for this emerging medical crisis. Lilly's diabetes portfolio accounts for one/fifth of the company's revenue, a figure analysts predict could more than double in the next four years. Lilly's expertise in both small and large molecule therapies and global diabetes sales force makes it one of the leading players in the fight against the global, diabetes epidemic.

A-2

Application of Primary Islet Cultures in Diabetes Research. M. B. BRENNER. Lilly Research Laboratories, Diabetes Pharmacology Hamburg, Essener Bogen 7, 22419 Hamburg, GERMANY. E-mail: Brenner_Martin@Lilly.com

Cell-based assays with tumor-derived insulin secreting cells are heavily used high-throughput tools in drug discovery. Most of the cell-lines in question are easy to culture and use. Although pancreatic islets are complicated to isolate, difficult to culture and don't allow a noteworthy throughput they provide several advantages over tumor-derived cells. Each pancreatic islet represents a fully functional miniature endocrine organ consisting of different cell types, arranged in a specific way that allows the islet to secrete hormones in a physiological manner. In pancreatic islets the glucose dependency and the typical biphasic insulin secretion pattern are preserved and can be studied in static incubation and perifusion experiments. Furthermore, the use of islets from diabetic animal models in perifusion studies allows to examine the pathology of insulin secretion. Additionally, pancreatic islets are better suited to evaluate anti-apoptotic effects of novel drugs than immortalized cell lines. They furthermore provide valuable insights into changes in gene expression caused either by the treatment of animals with a drug or after in vitro incubation with compounds. In conclusion, although pancreatic islets do not allow high throughput screening they can provide important additional information about the mechanism of action and physiological relevance of novel compounds.

A-3

In Vitro Models and Their Automation for Use in Screening Compounds and Selecting Lead Therapeutic Candidates for Diabetes Treatment. S. D. KAHL. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285. E-mail: skahl@lilly.com

Cell-based assays have been routinely used for identifying compounds which have potential utility for diabetic therapy. These include insulin secretion, insulin sensitivity and other glucose homeostatic mechanisms. Although typically more complex than biochemical assays, in vitro cell-based functional assays represent versatile and unique tools in a pre-clinical drug discovery strategy. They can assist with understanding mechanism of action and permeability as well as a possible predictor of in vivo activity. The measurement of second messenger response for a G-protein coupled receptor (GPCR), an important disease target class, is one example of how cell-based assays can be used to identify and characterize

initial molecules of therapeutic interest. However, developing and maintaining a robust and reproducible assay over a period of time can be challenging. This presentation will focus on the steps involved in optimization and statistical validation of representative in vitro cell-based functional assays that support several GPCR targets in the diabetes drug discovery portfolio at Eli Lilly and Company. Choice of detection technology, implementation of automated equipment and assay quality control analysis methods will be also be discussed.

A-4

The Application of In Vitro Cell-based Models for Quality Assurance in the Manufacture of Diabetes Therapeutics. B. S. PAREKH. Eli Lilly and Co., Lilly Technology Center, Indianapolis, IN 46221. E-mail: parekhbs@lilly.com

Biotechnology therapeutics, such as humanized monoclonal antibodies and other protein therapeutics, represent a rapidly growing class of molecules being developed for a wide range of diseases such as diabetes and cancer. Together with physico-chemical assays, cell-based assays represent a powerful orthogonal approach to assessing the quality of the product as well as the processes utilized to manufacture it. Specifically, bioassays are used to support process development and the release and characterization of clinical trial materials as well as the release of commercial therapeutics. Thus, bioassays need to be optimized and qualified/validated to be used as quantitative analytical tools. Strategies for the development and utility of bioassays to support the process and clinical development of bio-therapeutics for diabetes will be discussed.

A-5

Why It is Important to Authenticate and Characterize Your Cell Lines. YVONNE REID. American Type Culture Collection, Cell Biology Program, 10801 University Boulevard, Manassass, VA 20110-2209. Email: yreid@atcc.org

Animal cell lines are widely used by scientists in many diverse disciplines such as cell biology,gene therapy, gene expression and gene mapping. This wide application of cell lines usage hasled an increase in the number of misidentified and cross-contaminated cell lines. Yet scientistsignore the problem and continue to use cell lines that are not authenticated or characterized. Thispresentation provides a comprehensive approach to the authentication and characterization of animal cell lines.

A-6

Development of an Optimized STR DNA Marker System for Cell Identification and Contamination Detection. JOHN WATSON. Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711. Email: john.watson@promega.com

Cell based research is dependent on maintaining the genetic integrity of the cell lines used. Contamination of the cell lines of interest by other cell lines, mouse feeder cells, or mycoplasma can lead to erroneous conclusions such as the mis-identification of cell lines which recently occurred in Korea. In this study we describe a new system specifically designed to positively identify human cell line origins. In addition, the technology enables identification of mouse cell and/or mycoplasma contamination in a single test. The assay uses a multiplexed PCR protocol that incorporates 9 human tetranucleotide short tandem repeats (STR), a locus specific to human gender, a single mouse locus and a pan-mycoplasma locus. By incorporating 9 human loci we are able to identify the human source of the cell line to a certainty of ~1 in 10,000,000. The mouse primers will identify any mouse cell line contamination of the human cell line greater than 1%. The mycoplasma primers were designed to detect the majority of potential contaminating mycoplasma species. In order to validate the new technology we compared it to an existing STR kit that contains 16 loci and found that the extra resolving power of the 16 loci kit did not significantly benefit identification of the human cell lines. By using fewer human loci the assay can incorporate the additional mouse and mycoplasma amplicons in a single PCR amplification. Routine incorporation of STR typing prior to publication or cell line transfer should help assure the integrity of cell lines used for basic research and clinical therapies.

A-7

Cell Line Authentication by Isoenzyme Analysis: Lessons Learned in the Biotechnology Industry. RAYMOND W. NIMS and Christopher J. Herbstritt. BioReliance, 14920 Broschart Road, Rockville, MD 20850. Email: rnims@amgen.com

In the highly regulated biotechnology industry, cell line identification is required for all cell substrates employed for the manufacture of biologics. A result of this, and other Good Manufacturing Processes (GMP) requirements associated with the use of cells, has been that the incidence of mis-identification in the biologics manufacturing industry is extremely low. We

reported in 2005 that only 2 cases of mis-identification, and 2 cases of interspecies co-cultivation (not counting human cells grown on mouse feeder cells) were detected over a 10-year period in which over 900 individual cell samples were identified using isoenzyme analysis (Innovative Chemistry's AuthentiKit®). It must be made clear that the incidence mentioned above is for cells used in a *GMP* setting. It is not possible from our data to estimate the incidence of cell line cross-contamination and mis-identification for research settings, even those within the biotechnology industry. The technique as used in the GMP setting will be discussed. Recommended use of the technique for research settings as a means of reducing the occurrence of cell line misidentification and cross contamination will also be presented.

A-8

Challenges and Future Directions for Detection of Ricin and Other Related Toxins. VIPIN K. RASTOGI[1], Lalena Wallace[1], and Kerri Spitz[2]. [1]BioDefense Team, Research & Technology Directorate, US Army - ECBC, APG, MD 21010 and [2]ORISE, APG, MD 21010. Email: vipin.rastogi@us.army.mil

Recent use of ricin toxin as a biological weapon highlighted a need for a fast, reliable and sensitive approach for its detection. Ricin is a potent toxin, making up 1–5% by seed weight of the castor beans. The LD_{50} in laboratory mice is 3–5 μg/kg and ~60 hours inhalation exposure is lethal. Ricin is a hetero-dimeric ribosome-inactivating protein (66 kD in size) with two subunits, chain A (262 amino acids in length, 32 kD) and chain B (267 amino acids in length, 34 kD). Chain B binds to the mammalian cell receptors enabling the entry of chain A, where the latter acts as an enzyme catalyzing cleavage and release of adenine base from the ribosomal RNA. This inactivation results in loss of ribosome assembly and protein synthesis. In this presentation, different approaches for detection of ricin will be reviewed. Broadly, the detection approaches can be grouped in two categories: 1) surface or structure-based, i.e. immuno-based or analytical methods coupled with mass spectrometry; and 2) function-based, i.e. in vitro protein inhibition, release of adenine from intact ribosomal RNA, or in vivo cell toxicity detected by colorimetric signal. Even though the detection limits are generally low by first approach and range between micrograms to picograms levels, the biological activity of such ricin toxin remains questionable. The strengths and shortcomings of both approaches will be highlighted. Furthermore, future directions towards developing an activity-based in vivo toxicity assay will be discussed. Finally, planned efforts in decontamination of ricin toxin on building interior surfaces will be presented.

A-9

Plants as Sensors for Toxic Industrial Chemicals and Munitions: A Feasibility Analysis. MICHAEL K. DEYHOLOS[1], Derek Rogge[2], Benoit Rivard[2], and Anthony A. Faust[3]. [1]University of Alberta, Department of Biological Sciences and [2]Department of Earth and Atmospheric Sciences, Edmonton, Alberta, CANADA T6G 2E9 and [3]Defence R&D Canada - Suffield Box 4000 Station Main, Medicine Hat, Alberta, CANADA T1A 8K6. Email: deyholos@ualberta.ca

As self-replicating, self-powered entities, plants are attractive systems for potential applications in chemical monitoring, including the detection of toxic chemicals or explosives in air or soil. We have evaluated both natural and transgenic plants for their suitability as sensors in humanitarian and strategic contexts. In a first series of experiments, we have exposed three species of plants (poplar, wheat, oilseed rape) to various concentrations of NH_3, Cl_2, SO_2, H_2S, and HCN gases and measured the effects of these gases on basic physiological parameters and on the reflectance spectra. We have compared these responses with the changes induced by developmental effects and environmental stresses that might be encountered naturally. These data form the basis of an ongoing study on the feasibility of detecting toxic gas releases using remote hyperspectral imaging. In a second series of experiments, we are designing and evaluating components of genetic circuits that might be used in transgenic plants for the detection of certain chemicals, especially 2,4,6-trinitrotoluene (TNT) and its derivatives that might accumulate around land mines. We are studying three types of components of such a system simultaneously: i) a root-localized sensor/receptor of TNT and its derivates, ii) a stem-localized signal transducer and iii) a shoot-localized reporter. We have concluded that engineered bacterial proteins and RNA-based receptors are superior to endogenous promoters as candidate sensors, and pigment-altering reporter systems in shoots have already been reported by ourselves and others. Refining the sensitivity, specificity, and practicality of these components, and linking them by a root-to-shoot signal transduction system are the current objectives of our research efforts. This research is funded by the Department of National Defence (Canada), and by the Canadian Center for Mine Action Technology.

A-10

Development of a Cell-based Toxicity Sensor for Drinking Water Protection. M. W. WIDDER[1], T. Curtis[2], L. Romeo[2], L. Brennan[3], and W. van der Schalie[1]. [1]U.S. Army Center for Environmental Health Research, Fort Detrick, MD 21702; [2]Agave BioSystems, Ithaca, NY 14850; and [3]SAIC, Fort Detrick, MD 21702. Email: mark.widder@us.army.mil

The U.S. Army Center for Environmental Health Research is developing an Environmental Sentinel Biomonitor (ESB) system platform that includes toxicity sensors capable of rapidly identifying toxicity associated with a broad range of industrial and agricultural chemicals in drinking water. Of the toxicity sensors evaluated to date, the best overall performance has been by a sensor that measures electrical impedance across live cells (Agave BioSystems, Ithaca, NY). Several advancements have been made to the cell impedance system to improve its performance. A closed-system fluidic microelectrode array has been developed for improved long-term cell storage and maintenance which allows for increased portability for field testing needs. Increased sensitivity to toxic chemicals has been achieved through the selection of optimized cell types for use with the cell impedance measurement technology and through improved statistical analysis techniques. An automated cell maintenance system will improve field portability and ease of use, and simplify long term storage of the cell impedance sensors. The cell impedance sensor will be included in a prototype ESB system scheduled for completion in 2008.

A-11

New Look at an Old Procedure: Tissue Dissociation in the 21st Century. ROBERT MCCARTHY. VitaCyte LLC, 1102 Stadium Drive, Indianapolis, IN 46202. Email: rmccarthy@indy.rr.com

Tissue dissociation using “crude collagenase” has been used for over 40 years with minimal improvements to the technology. An R&D group at Boehringer Mannheim in Indianapolis in the early 1990’s defined the key enzymes required for human islet isolation. This led to the development of the Liberase Purified Enzyme Blend Product line that contained purified collagenase and neutral protease enzymes. The Liberase HI product significantly increased the yield and quality of islets when compared to those islet preparations prepared using crude collagenase. This reagent improvement was, in part responsible for the success of the Edmonton Protocol that used islet transplantation to treat adult type 1 diabetic patients who were disabled by refractory hypoglycemia. The lessons learned from using purified proteases for tissue dissociation will be reviewed and a scenario presented why higher quality primary cells will become increasingly important as we enter into the post genomics/proteomics/metabolomics era of life science research.

A-12

State-of-the-art in Flow Cytometry: 2007. J. PAUL ROBINSON. Purdue University Cytometry Laboratories Bindley Bioscience Center, 1203 West State Street, Discovery Park, Purdue University, West Lafayette, IN 47907-2057. Email: jpr@flowcyt.cyto.purdue.edu

This presentation will outline the current capabilities of flow cytometry including technological advances as well as new applications. As the field becomes more engaged in biotechnology and as advanced computation embeds itself within the field of cell analysis, there are many changes from the days of one and two color analysis. Advances in multispectral analysis as well as new spectral probes and images based cytometers are changing the landscape of clinical and research laboratories.

A-13

Ambient Mass Spectrometry Applied to Biological Problems. R. GRAHAM COOKS. Purdue University, Department of Chemistry, 560 Oval Drive, West Lafayette, IN 47907-2084. Email: cooks@purdue.edu

This talk introduces the method of desorption electrospray ionization (DESI) mass spectrometry, a procedure in which samples are examined outside of the mass spectrometer, by spraying them with a fine mist of charged microdroplets. The experiment gives very rapid responses, and is highly sensitive and specific. Applications are demonstrated to characterization of whole bacteria, to serum and urine analysis for endogenous compounds, to metabolomics and to tissue imaging to locate diseased tissue.

A-14

Outsourcing Innovation in the Pharmaceutical Industry, Rise of Business Ecosystems. ROBERT MCCARTHY. VitaCyte LLC, 1102 Stadium Drive, Indianapolis, IN 46202. Email: rmccarthy@indy.rr.com

Substantial R&D investment by the pharmaceutical industry over the last twelve years has led to a decrease in the introduction of new drugs onto the market. A white paper published in 2004 by the FDA (Innovation or Stagnation) presented an analysis of the “pipeline problem”. A key

conclusion was that not enough new tools are being developed to test the safety and effectiveness of new products. Focus in this area of "critical path R&D" will lead to decreased costs and reduced time for new product development. Several leading pharmaceutical firms have tackled this issue by looking outside to find innovative solutions to R&D problems. Illustrations will be presented to highlight this shift, leading to the rise of business ecosystems that are rapidly becoming an extended enterprise innovation network. The implications of this change for the viability of technically focused firms will be reviewed.

A-15

Stem Cells in Regenerative Medicine. DEBRA GREGA. Center for Stem Cell and Regenerative Medicine, National Center for Regenerative Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-7284. Email: debra.grega@case.edu

The use of stem cells in regenerative medicine has caught the imagination of the world with its promise to rebuild broken bodies. The debate about embryonic vs. adult-derived stem cells has become a focal point in the public and scientific communities. However, there are well accepted stem cell therapies being used today to treat a number of disorders. Regardless of the source, stem cell therapies have common issues for routine therapeutic use. We will briefly explore some of the hurdles to the widespread use of such cellular therapies. Additionally we will briefly survey the current and near-future disease areas that appear appropriate for cell-based therapeutics either alone or as composite therapies consisting of cells, scaffolds, innovative devices and stimulatory modules, and biologics that support regeneration.

A-16

Commercializing Hematopoietic Reconstitution Using Adult Cord Blood Stem/Progenitor Cells Expanded in Culture: Challenges and Opportunities for Industry. PAUL A. HYSLOP. EndGenitor Technologies, Inc., 351 W. 10th St, IN 46202, Email: physlop@iupui.edu

Over 18 years ago, Gluckman and colleagues first demonstration that hematopoietic reconstitution could be accomplished using HLA-matched sibling cord blood. Since that time, implementation of cord blood banks, pioneered by scientists at the New York Blood Center, led to the successful demonstration that the HLA-typed mononuclear cells (MNC) isolated from cord bloods could be cryopreserved and banked for transplantation. As the science of adult stem cell research has evolved, several studies have been documented that demonstrate that $CD34^+$ cells containing the hematopoietic stem/progenitor cell population, isolated from cord blood MNC, has the potential to be expanded in vitro for therapeutic use. Development of an "off the-shelf" cryopreserved, HLA-typed, standardized therapeutic unit of banked $CD34^+$ cells from cord blood has become a major research focus. In this presentation, progress towards successful commercialization of expanded $CD34^+$ cells for unrelated hematopoietic reconstitution, will be discussed, highlighting both recent successes and the remaining obstacles and challenges to successful product development.

A-17

Stem Cells in Cardiovascular Disease. KEITH L. MARCH. Indiana Center for Vascular Biology and Medicine, Indiana University, 975 West Walnut Street, IB 441, Indianapolis, IN 46202. Email: kmarch@iupui.edu

This presentation will provide an understanding of some readily available adult stem and progenitor cells, and key experimental as well as clinical data that is emerging in Indiana about their potential for practical therapeutic utility in several important diseases. These cells may be obtained in abundance from fat as well as from bone marrow, umbilical cord, and other sources. Preclinical information will be discussed in some detail regarding the utility of adipose (fat)-derived stem cells. From a clinical perspective, the presentation will describe the recent completion at Indiana University of the first FDA-approved, Phase 1 clinical trial of bone marrow-derived cells for patients with severe peripheral vascular disease and threatened amputation.

A-18

Introduction to Scaffolds for 3-D Cell Culture. PAUL J. PRICE. D-Finitive Cell Technologies, 1521 Red Drum Road, Mount Pleasant, SC 29466. Email: p.price05@comcast.net

Numerous scaffolding materials are being used to grow cells as proto-tissues. Which scaffold to use is dependent on the desired results. This introductory talk will present the role of the scaffold, what is currently available and how to select the right scaffold.

A-19

3D Spheroid Cell Culture using Alginate Sponge Scaffolding. RICHARD FIKE. Invitrogen Corp., R&D Department, 3175 Staley Road, Grand Island, NY 14072. Email: Richard.Fike@invitrogen.com

3D cell culture is now accepted as yielding more in vivo-relevant information than 2D polystyrene flat culture. Spheroid formation is a subset of 3D cell culture where cells maximally interact with each other, resulting in formation of in vivo-like tissue and organ structures. Extensive use of this format is now well accepted in tumor biology where the Multicellular Tumor Spheroid (MCTS) assay yields valuable information on chemosensitivity and biology of specific cancers while hepatocyte and cardiomyocyte spheroid culture provides platforms for understanding differentiation and morphogenesis. The alginate sponge format is easy to use, simply requiring cells to be inoculated directly into the sponge. Several inoculation protocols will be discussed. Analytical options include 1) in situ manipulations, 2) fixing, embedding and sectioning of intact sponges containing spheroids, 3) dissolution of sponges to yield spheroids themselves, and 4) dissociation of spheroids to yield individual cells for analysis or further culture. Differences between 2D and spheroid 3D cell culture will be presented. HepG2 â€“C3A hepatocarcinoma cells readily form spheroids in the alginate sponge that result in a greater than 2X increase in CYP1A2 enzyme induction compared to 2D culture. Data will also be presented showing that fresh hepatocytes and cardiomyocytes attain in vivo-like characteristics and that stem cell embryoid bodies differentiate in alginate sponges.

A-20

Tissue Engineering and 3-D Cell Culture Using Hyaluronan Based Hydrogels. ANNA SCOTT[1], Xiao Zheng Shu[2], Guanghui Yang[2], Terry Tandeski[2], Yanchun Liu[2], Glenn D. Prestwich[2]. [1]Glycosan BioSystems Inc., 675 Arapeen Way, Suite 302, Salt Lake City, UT 84108 and [2]Center for Therapeutic Biomaterials, University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, UT 84108. Email: jascott@glycosan.com

We have recently developed a novel approach to the creation of a fully synthetic, covalently crosslinked extracellular matrix known as Extracel™. Extracel™ can be crosslinked under ambient, physiological conditions in situ in the presence of cells to provide an injectable cell-seeded hydrogel for tissue repair in vivo or three-dimensional (3-D) cell culture in vitro. The hydrogel is composed of thiol-modified hyaluronan, thiol-modified gelatin (denatured collagen), and polyethylene glycol diacrylate (biocompatible, polyvalent electrophile that reacts with the thiol residues to crosslink the hydrogel). Extracel™ hydrogels and sponges (lyophilized hydrogels) support in vivo growth of healthy, cellularized tissues and in vitro growth of primary human hepatocytes, human dermal fibroblasts, mesenchymal stem cells and many other primary cells and cell lines. For tissue engineering, Extracel™ impregnated with cells dramatically improves repair of bone and cartilage defects and aids in the regeneration of functional liver tissue.

A-21

Fish Cell Lines as Biosensors of Aquatic Environments. LUCY E. J. LEE[1], Kristin Schirmer[2], and Niels C. Bols[3]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, Ontario,CANADA; [2]Dept. of Cell Toxicology, UFZ Centre for Environmental Research Leipzig-Halle, Leipzig, GERMANY; and [3]Department of Biology, University of Waterloo, Waterloo, Ontario, CANADA. Email: llee@wlu.ca

Many chemicals, natural or manmade, accidentally or purposely discharged, end up in waterbodies, causing adverse effects to the aquatic biota and ultimately to humans. Thereforemonitoring of water sources for toxicants or potential toxicants is of high priority, but although analytical instruments are available that can detect traces of various contaminants, no machinecan actually determine whether compounds are toxic or not to living organisms. Regulatorytesting of industrial effluents often requires fish lethality assays, which can be laborious andcostly. Thus, alternative evaluation methods that are simpler and cheaper but still sensitive andrelevant have long been sought for the testing of effluents or of individual chemicals. This isespecially relevant when new synthetic chemicals are discharged annually to water bodies aroundthe world. We present here, over twenty years of research with fish cell lines as possiblealternatives to animal testing and as useful biosensors for aquatic environments. The maintenanceand handling of fish cell lines is simpler than for mammalian cell lines, an aquatic animal facilityis not needed and quantitative measures of toxicity can be obtained rapidly, reproducibly andeffectively. Sample applications with selected chemicals, industrial effluents, oil sands process affected waters, and more recently with nanoparticles will be presented. Advantages over the useof mammalian cell lines, such as the ability to directly expose cells to effluents without the needof extracting chemicals will be discussed, as well as presenting our recent work with newlydeveloped fish cell lines that express origin-specific, differentiated functions that can be usefulfor elucidating mechanisms of action of toxicants.