

Animal Symposia

A-1

Tissue Engineering and Ex Vivo Tissue Test Systems. K.J. L. BURG1 and D. Dréau2. 1Department of Bioengineering and Institute for Biological Interfaces of Engineering, Clemson University, Clemson, SC 29631 and 2Department of Biology, University of North Carolina at Charlotte, Charlotte, NC 28223. Email: kburg@clemson.edu

Regenerative medicine is the field at the intersection of science, engineering, and medicine that encompasses the creation of biological implants to augment or replace damaged tissues or organs, often using a biomaterial template or scaffold. The initial promise of tissue engineering was that a damaged tissue might be replaced using a few cells derived from the patient; e.g., a liver might be grown for a recipient rather than transplanted from a donor. This early concept of developing tissue for reconstructive purposes has evolved to include the construction of test tissues systems for therapeutic development, developmental cell biology studies, and disease prevention. Regardless of the end goal, the viability of a tissue-engineered product relies on cell-biomaterial interaction and the design of an appropriate 3-D in vitro environment. This presentation will overview approaches and data obtained toward the development of test systems for breast cancer diagnosis and tissue engineered implants for breast tissue reconstruction. In addition, the importance of an interdisciplinary team approach to the successful construction of in vitro 3-D breast tissue systems will be highlighted. (The presentation will overview work supported by grants from the Department of Defense Era of Hope program and the National Science Foundation Emerging Frontiers in Research and Innovation program.)

A-2

Injectable Hydrogels for Brain Tissue Regeneration After Traumatic Brain Injury (TBI). NING ZHANG. Clemson-MUSC Joint Bioengineering Program, Department of Bioengineering, Clemson University, 173 Ashley Ave. BSB#601, Charleston, SC 29425. Email: nzhang@clemson.edu, zhangn@musc.edu

Following TBI, injury and inflammation of brain tissue result in the swelling of various structures within a finite and

constricting space. In addition to the mechanical injury to the cells, the elevated pressure due to brain tissue swelling leads to the release of compounds that cause secondary injury to healthy brain tissue beyond the primary insult. Persistent cell dysfunction and poor neural regenerative capabilities at the TBI site and beyond lead to the formation of a lesion defect or cavity that is associated with prolonged neurological impairment. Conventional treatments for TBI have been focused on managing the primary injury using hypothermia, osmotic therapy, and decompressive craniectomy, as well as neuroprotection with pharmacological agents to reduce the secondary damage. Despite the benefit in small numbers of patients, none of these treatments have been translated into clear improvements in the mortality and neurological outcome. This is perhaps due to the inability of these treatments to repopulate the lesion with functional neural cells. In alignment with this notion, neural transplantation strategies have been tested to reconstruct the lesion cavity. Despite its efficacy in providing sustained functional recovery in some CNS diseases, neural transplantation for TBI repair has had limited success, due to poor donor cell survival and functionality at the lesion site. In particular, the ongoing tissue inflammation and scarring at the lesion site and the lack of any supportive tissue structure and vasculatures within the cavity present a hostile environment that jeopardizes the survival of transplanted cells. For cell replacement at the TBI lesion cavity, there is a critical need to pre-condition the lesion site with vasculature network to support subsequently arriving neural cells/precursors. To this end, we have developed an *in-situ* crosslinking hydrogel with similar mechanical property to that of native brain tissue and cell adhesive motifs. We have demonstrated for the first time the formation of a well-structured vasculature network within the injected hydrogel at the TBI lesion cavity without the use of angiogenic growth factors. Further, to improve functional recovery, we transplanted human neural stem cells with neural differentiation factors to the TBI lesion using our hydrogel as carrier and significant functional recovery was found after 8 wks of treatment.

A-4

Transfection of Marine Sponge Cells to Produce a Cell Line. SHIRLEY A. POMPONI. Harbor Branch Oceano-

graphic Institute, Florida Atlantic University, 5600 U.S. 1 North, Fort Pierce, FL. Email: spomponi@hboi.fau.edu

Sponges are a major source of marine-derived chemicals with biomedical applications, but the metabolites are often in trace amounts too low for commercial production. In vitro sponge cell culture may provide a well-defined and controllable environment for the production of chemicals and other bioproducts of interest. Using a liposome-mediated transformation system (Lipofectamine), we have successfully introduced an expression vector containing human telomerase reverse transcriptase (hTERT) into cell suspensions of the marine sponges, *Axinella corrugata* and *Xestospongia muta*, and have obtained transient expression of the gene. Transfection was optimized by using a concentration gradient of transforming agent to a constant number of cells. Expression of hTERT was verified by extraction and PCR analysis of DNA from the treated cells. These results lay the groundwork for establishing an immortalized sponge cell line.

A-5

Stem Cells from Marine Invertebrates – Perspectives and Prospective. BARUCH RINKEVICH. National Institute of Oceanography, Haifa, ISRAEL. Email: buki@ocean.org.il

This presentation analyzes the activities in the field of marine invertebrate cell cultures during the last two decades (1988-2008) by comparing the outcomes of former years with recent studies (2005-2008). The last two decades have witnessed variable activities, by numerous laboratories, to develop cell cultures from marine organisms. However, while attempts to develop cell cultures from many marine invertebrate taxa date back about a century, for reasons that remain obscure, all endeavors to develop cell cultures from marine invertebrates have been ineffective so far despite the acknowledged need for cell cultures from species that are important in aquaculture or for the pharmaceutical industry. Attempts to develop cell cultures from marine organisms included the development of unique techniques (such as methodologies and specific culture media), the choice of best cell/tissue/organ source for the study, the use of tissue fragments and non-adherent cells, the validation of in vitro techniques and the need for cryopreservation techniques for cells. The review will further present trends in the research, such as the attempts to improve cell culture methodologies, the increased interest in employing already existing methodologies in applied aspects, long-term vs. short-term cultures, the use of model organisms and taxa and the recent employment of cellular, genomic and proteomic tools.

A-6

Crustacean and their Pathogens: Developing Susceptible Host Cell Lines and Alternative Approaches. KAREN G. BURNETT. Grice Marine Laboratory, 205 Fort Johnson Rd., and Hollings Marine Laboratory, 331 Fort Johnson Rd., Charleston, SC. Email: burnettk@cofc.edu

Despite extensive effort for more than forty years, the goal of establishing continuous cell lines from marine crustaceans remains largely elusive. The demand is great, driven in large part by the need for in vitro systems in which to study pathogens of cultured shrimp species, such as *Penaeus monodon* and *Litopenaeus vannamei*, which have high economic value around the world. Recent efforts have focused on transfecting primary cells with genes that suppress apoptosis or on transforming primary cells with pantropic viruses. There are reports of established crustacean cell lines that are susceptible to shrimp viruses, but these have yet to become generally available. In the absence of in vitro systems amenable for studying disease pathogens and immune defense, in vivo approaches have provided some understanding of how pathogen and their hosts. We have transfected the opportunistic bacterial pathogen, *Vibrio campbelli*, with a stable plasmid expressing GFP and resistance to antibiotics kanamycin and chloramphenicol. Using standard microbiological techniques and quantitative PCR, we have monitored the tissue distribution and degradation of these bacteria in whole animals, generating evidence of the differing roles of these tissues in immune defense against bacteria, and how these defenses are impacted by environmental stressors such as hypoxia and hypercapnia (supported by NSF Awards 0725245 and 0212921.)

A-7

Toxicity Pathways, In Vitro Assays, and Computational Cell Biology: Using the Best Science for Toxicity Testing and Risk Assessment. MELVIN E. ANDERSEN. The Hamner Institutes for Health Sciences, P.O. Box 12137, 6 Davis Drive, Research Triangle Park, NC. Email: mandersen@thehamner.org

The 2007 NAS report, “Toxicity Testing in the 21st Century – A Vision and A Strategy”, outlined new initiatives for using *in vitro* methods to evaluate toxicity pathway responses, for applying computational systems models for assessing dose response of pathway perturbations, and for grounding test-tube to human extrapolations in in vitro-in vivo dosimetry. On-going dialog over the past two years among many interested parties has extended and re-shaped many ideas about steps required to implement the

NAS vision. In this talk, the speaker, a member of the NAS Toxicity Testing committee, describes key report recommendations for in vitro testing of toxicity pathways and for the development of the interpretive tools necessary for using these in vitro results in risk and safety assessments. Coordinate progress in assay design/validation and computational modeling of the cell networks queried by particular assays will guide use of in vitro test results in more quantitative, human health risk assessments.

A-8

Mechanistic Insight into Drug-Induced Organ Injury with Comparisons of Animal and Human Tissue. A. E. M. VICKERS. Drug Safety Evaluation, Allergan, Inc., Irvine, CA 92612. Email: vickers_alison@allergan.com

Predicting the relevance of drug induced organ injury for human, identified from animal studies, is addressed with organotypic cultures via comparisons of animal and human tissue. The cellular pathways underlying the mechanisms of target organ injury are generally comprised of biochemical networks representing a myriad of cell-cell and cell-matrix interactions. The application of organotypic cultures combined with gene expression profiling plus biochemical measurements characterizes the molecular mechanisms of drug action on the activation of cell death and survival pathways including oxidative stress, inflammation, apoptosis, and repair, with the goal to identify key pathways that are causal to organ dysfunction and injury. These technology advances plus the application of human tissue models to define the clinical relevance of side-effects and biomarkers is redefining the scope of translational research in toxicology. The increased utilization of such models earlier to support drug selection adds value to the safety decision process. Examples will be presented which elucidate the mechanisms of drug-induced organ injury, define species susceptibility to organ injury, and aid in characterizing human response. One example, drug induced mitochondrial injury demonstrates the interplay of cells in liver slices by the induction of oxidative stress, increased apoptosis and inflammation. A second example is a hemolysis model, comprised of whole blood co-cultured with a liver slice, to evaluate time- and concentration-dependent toxicity on red blood cells. A third example reveals that drug retention in thyroid is associated with inhibition of thyroid peroxidase function and potentially thyroid hormone synthesis. In each of these examples rat and human response is compared.

A-9

Knowledge Profile Approach: Insights into Drug Action and Toxicity Mechanisms. NIKOLAI DARASELIA. Ariadne

Genomics, Rockville, MD. Email: nikolai@ariadnegenomics.com

Providing a rich context for experimental data promises to offer new insights into mechanisms of compound action and toxicity. Employing a resource of millions of findings gleaned from a broad corpus of biomedical literature in which to evaluate genome-wide experimental data can highlight specific molecules otherwise easily missed. The challenge to use this large amount of data in decision-making can be met using appropriate hypothesis testing. Using the proprietary high-content linguistics tool MedScan we compiled a database of knowledge profiles associated with different diseases and small molecule effects by extracting the information from scientific literature. Different approaches towards reconstructing individual pathways or cascades from the resulting ChemEffect™ knowledge-base and from microarray data will be described. Our visualization software Pathway Studio is capable of systematically mining this database for knowledge on existing drugs/drug candidates garnered from published findings, and is shown to help in tasks as diverse as 1) the identification of potential novel components of glioblastoma pathway and suggesting a new application for a known agent to inhibit such pathway, and 2) hypothesizing a mechanism behind drug induced cholestasis and suggesting a biomarker candidate independently validated in other studies.

A-10

Using Gene Expression and Pathway Analysis for Efficacy and Toxicity Assessment. EUGENE ELMORE^{1,2}, J. Leslie Redpath^{1,2}, and Vernon E. Steele³. ¹Dept of Radiation Oncology, University of California, Irvine, CA 92697; ²Chao Family Comprehensive Cancer Center, University of California, Irvine, CA 92697; and ³Division of Cancer Prevention, NCI, 6130 Executive Blvd, Bethesda, MD 20892-7322. Email: eelmore@uci.edu

The determination of potential clinical agent toxicity and efficacy can be assessed using gene expression and pathway analysis in cultured human cells. Data from two potential cancer preventive agents, celecoxib and NO-aspirin, will be used to illustrate the application of modern tools for analysis of the massive amounts of data generated in gene expression studies. The tools are used to characterize the fingerprints of patterns of gene expression data and the implications as to potential function. The identification of changes in critical pathways can be used to identify changes that could result in potential efficacy, changes in functional regulation, and toxicity or adverse events. Data with celecoxib, a Cox-2 inhibitor, will be presented to show

that achievable plasma concentrations can be used to identify concentration and time related changes that are relevant to potential efficacy. The data indicate that continuous exposure at clinically-relevant concentrations can induce apoptosis and inhibit cellular growth in human colon polyp cells. In addition, the issue of "more is better" will be discussed. NO-aspirin, an NO donating NSAID, was evaluated at a concentration achievable in plasma. The aspirin moiety was able to inhibit Cox-2, which would suggest potential cancer preventive efficacy. The gene expression changes following NO-aspirin treatment suggested a role in apoptosis, WNT signaling, and the P40 MAP kinase pathway. For each agent, the potential clinical efficacy as well as potential for adverse events will be discussed. Supported by NCI contract #N01-CN-43300.

A-11

3-D Tissue Models in Contract Research: Points to Consider for Efficacy, Product Development, or Regulatory Testing Programs. H. A. RAABE, Institute for In Vitro Sciences, Inc., 30 West Watkins Mill Road, Suite 100, Gaithersburg, MD 20878. Email: hraabe@iivs.org

Three-dimensional tissue constructs are often used in contract research testing for in vitro modeling of tissues and organs. The CRO's Primary Investigator (PI) needs to weigh a number of considerations in selecting the appropriate tissue model depending upon the goals of the project. There may be varying requirements for the specificity and relevance of the tissue to be modeled. The PI may either desire a well-defined tissue model relevant to a regulatory endpoint, or alternatively a custom model designed to enhance novel research endpoints. Additionally, the PI must consider the reliability of the tissue construct, to judge whether tissue reproducibility within a trial, as well as lot-to-lot is important. Other considerations include tissue costs, whether tissues are routinely and readily available, and whether the manufacturer can co-operate in research goals. Pharmaceutical research and product efficacy activities generally depend upon custom tissue models, whereas in product development and safety screening or regulatory testing well-defined, relevant and reliable models are critical. Ostensibly, the commercial tissue manufacturer and the academic laboratory each have specific unique organizational characteristics which may be ideally suited to the varying needs for efficacy, product development, or regulatory testing. The academic laboratory may be more adept at producing small custom batches of tissues for a limited number of research trials, while the mature manufacturing organization can apply its considerable resources to assure a continuous line of cost-effective, highly reproducible tissue constructs for safety and regula-

tory testing needs. Based upon these considerations, the PI has a number of options available to best meet the client's specific goals.

A-12

Customized 3D Human Tissues for Discovery. C. EGLES and J. A. Garlick. Division of Cancer Biology and Tissue Engineering, Department of Oral and Maxillofacial Pathology, Tufts University School of Dental Medicine, Boston, MA. Email: Christophe.egles@tufts.edu

Conventional basic science approaches to the dissection of complex biological systems have been based primarily on experimentation in 2D, monolayer culture systems. However, the power of these approaches to simulate biological processes in human tissues has been limited. In light of this, it is now widely accepted that cellular and tissue responses need to be studied in experimental systems that incorporate appropriate three-dimensional (3D) context and faithfully mimic their in vivo counterparts. Through the Center for Integrated Tissue Engineering (CITE) at Tufts University, we have developed in vitro human 3D tissue models, such as Human skin equivalents (HSEs), that recapitulate the complex tissue architecture present in human tissue in vivo. We have adapted HSEs to accelerate the discovery pipeline by generating 3D tissues that are customized to address specific questions in early-stage (target validation or interrogation) and late-stage discovery (safety and efficacy screening). In this presentation, we will describe several examples of collaborations between the CITE and research laboratories or pharmaceutical companies that demonstrate how 3D tissues now provide human tissues that: (1) enable investigation into the complex interplay between multiple cell and tissue types in biologically-meaningful, 3D tissue context, (2) provide a more comprehensive and global picture of how disease-associated pathways interact with their local microenvironment, and (3) serve as human, "pre-clinical" or "surrogate" customized tissues that set the stage for the translation of discoveries to the clinic through strategies that will allow rapid screening of human tissues.

A-13

Commercial Production of In Vitro Human Epithelial Models at MatTek Corporation: A Survey of Available Models and Applications. P. J. HAYDEN, J. Kubilus, S. Ayeahunie, Y. Kaluzhny, H. Kandarova, P. Kearney, M. Klausner, and J. Sheasgreen. MatTek Corporation, Ashland, MA 01721. Email: phayden@mattek.com

Techniques for in vitro reconstruction of 3-D epithelia from isolated human cells have become more refined and widely

utilized in recent years. These epithelial models are cultured on microporous membranes at an air-liquid interface to produce highly differentiated 3-D cultures that possess many of the physical and biochemical properties of in vivo epithelium. The in vivo-like characteristics and barrier properties allow application and evaluation of test chemicals, candidate therapeutic compounds and finished formulations in a more realistic manner compared to cultures submerged in media. Examples of models currently produced from normal (nontransformed) human epithelial cells by MatTek Corporation include dermal (EpiDerm™), ocular (EpiOcular™), airway (EpiAirway™), gingival (EpiGingival™), buccal (EpiOral™) and vaginal EpiVaginal™ epithelium. Second generation models provide additional levels of complexity by incorporating multiple cells types including fibroblasts (EpiDerm-FT™, EpiAirway-FT™, (EpiVaginal-FT™), melanocytes (MelanoDerm™) and/or dendritic cells (DC-100). The ultimate utility of in vitro epithelial culture models for widespread use in research applications and for regulatory purposes depends upon their performance and validation with respect to interlaboratory transferability, long-term reproducibility, and the extent to which in vitro results can be extrapolated to in vivo human results. An overview of the Good Manufacturing Production (GMP) process, long-term reproducibility data and status of validation programs for ocular irritation, dermal corrosion, irritation and phototoxicity, airway irritation and vaginal irritation will be presented.

A-14

Determining the Antimicrobial Activity of Bacteria Associated with a Caribbean Coral. MARIA I. VIZCAINO^{1,2}, Peter D. R. Moeller^{1,2,3}, and Pamela J. Morris^{1,2,4,5}. ¹Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC; ²Hollings Marine Laboratory, Charleston, SC; ³Toxin Chemistry, NOAA National Ocean Service, Charleston, SC; ⁴Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC; and ⁵Center for Coastal Environmental Health and Biomolecular Research, NOAA National Ocean Service, Charleston, SC. Email: vizcain@musc.edu, morrisp@musc.edu

Increased emergence of antimicrobial-resistant pathogenic bacteria has prompted the search for novel antibiotics in marine ecosystems, including coral reefs and the diverse microbial community associated with the corals' surface mucopolysaccharide layer (SML). Since marine bacteria can produce compounds that are chemically distinct from their terrestrial counterparts there is potential for the discovery of novel antibiotics, which serve as chemical

defenses against other bacteria, including marine pathogens. We have characterized the chemical ecology of the microbial community associated with the gorgonian coral *Pseudopterogorgia americana*, focusing on the hypothesis that *P. americana* SML-associated bacteria produce antibiotics against human and coral pathogens. For these studies, coral SML samples from three healthy and three diseased colonies were collected in March 2006 and the bacterial community was isolated on glycerol-amended seawater plates (GASW). We then developed an antimicrobial bioassay to screen 142 of the bacterial isolates for production of antimicrobial compounds against seven test strains known to be human and coral pathogens, which included *Escherichia coli*, *Bacillus subtilis*, *Kocuria rhizophila*, *Pseudomonas aeruginosa*, *Vibrio harveyi*, *Vibrio coralliilyticus*, and *Aspergillus sydowii*. Our results showed that 70% (99/142) of the coral isolates inhibited at least one test strain. Only one coral isolate, with 99% 16S rDNA similarity (1200 bp) to *Pseudovibrio* spp., inhibited all seven test strains. Future studies will include isolation and purification of the antimicrobial compounds from the *Pseudovibrio* spp. using bioassay-guided fractionations with high performance liquid chromatography (HPLC), and subsequent structure determination using NMR-based techniques. This work highlights the potential of coral-associated bacteria as sources of potentially novel bioactive marine natural compounds.

A-15

Online Verification of Cell Line Authenticity Using an International Reference Database. W. G. DIRKS¹, Y. Nakamura², H. G. Drexler¹, and H. Mizusawa³. ¹DSMZ - Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, GERMANY; ²RIKEN - Tsukuba Institute, Tsukuba, JAPAN; and ³JCRB - National Institute of Biomedical Innovation, Osaka, JAPAN. Email: wdi@dsmz.de

Neglecting guidelines for quality control and disregarding adequate cell culture technique are the key problems underlying misidentification and cross-contamination (CC) of cell lines. Recent publications demonstrate a growing scientific perception, that CC is the major risk for false scientific data and their misinterpretation. Unacceptable high percentages of bogus cell lines have triggered complaints among scientists calling for action in this regard, but no answers could be given so far to the simple question posed by students and post-docs whether or not cell lines are authentic. A way out of this dilemma could be a simple search engine for genetic profiles in the internet loaded by a database of Short Tandem Repeat (STR) reference data sets of human cell lines. The ideal case of

authentication is STR typing of donor/patient, primary tumor and the corresponding cell line sample via direct alignment of STR data. The normal case of an authentication procedure is a retrospective and multidisciplinary approach limited to cell banks applying strict characterization and approved identification programs during cell line accession. In collaboration with the Japanese Collection of Research Bioresources (JCRB), the RIKEN BioResource Center and the German Collection of Microorganisms and Cell Cultures (DSMZ) the databases of existing STR profiles have been fused together and have been examined for uniqueness. Unexpectedly, the fusion of the STR databases revealed 7 cell lines out of more than 3000 data sets (<0.2 %) as falsely deposited cell lines only. The presented database is the first international and most comprehensive STR DNA profile database of authenticated human cell lines, which will be linked to search engines for STR profiles on the homepages of JCRB, RIKEN and DSMZ.

A-16

Misidentification of Animal Cell Lines: Impact on Research. YVONNE A. REID. Cell Biology Program, ATCC, Manassas, VA. Email: yreid@atcc.org

Propagation of animal cell lines is widely used by scientist in many diverse disciplines such as basic cell biology, genetic mapping, gene expression, and gene therapy. Cell line authentication and characterization are crucial activities and yet are the most under appreciated tasks performed by most research scientists. The validity of conclusions drawn by researchers demands that consistent and unequivocal verification of cell line identity and function are precise. Yet over the years many cell lines have been shown to be misidentified or cross-contaminated, due in part, to lack of adequate authentication and characterization protocols available in previous years. Advances in new technologies have led to more accurate authentication and characterization of cell lines. The financial loss due to these errors is estimated in the millions of dollars. Accurate cell line authentication and characterization requires a comprehensive strategy which employs multiple complementary technologies which results in consistent and comparable data. An overview of the current technologies used to authenticate and characterize animal cell lines will be presented.

A-17

Simple Sequence Length Polymorphism (SSLP) Analysis to Determine the Strain from Which Mouse Cell Lines Are Derived. YUKIO NAKAMURA. Cell Engineering Division, RIKEN BioResource Center, Koyadai 3-1-1, Tsu-

kuba, Ibaraki, 305-0074, JAPAN. Email: yukionak@brc.riken.jp

Cross-contamination between cultured cell lines can result in the generation of erroneous scientific data. Hence, it is very important to eliminate cell lines that are of an origin different from that being claimed. Inter-species contamination can be detected by various established methods, such as karyotype and isozyme analyses. However, it has been impossible to detect intra-species cross-contamination prior to the development of technology to detect differences between cell lines at the molecular level. Recently, profiling of short tandem repeat (STR) polymorphisms has been established as a method for the analyses of human gene polymorphism. Gene profiling by STR polymorphism (STR profiling) is a simple and reliable method to identify individual human cell lines. Needless to say, the analysis similar to STR profiling is necessary for mouse cell lines. Thus, we investigated polymorphism of many marker genes among mouse strains and developed a panel of Simple Sequence Length Polymorphism (SSLP) markers that can be used for profiling genetic background of mouse strains. After searching, we selected 6 kinds of highly polymorphic marker that represent relatively large difference of PCR product sizes and therefore are distinguishable by using Gene Mapper System of ABI DNA Sequencer. The method we established enabled rapid, precise and large-scale surveillance of genetic background of mouse cell lines. I introduce the method and results.

A-18

Current Trends on the Identification and Propagation of Entomopathogenic Nematodes. S. PATRICIA STOCK. Dept. Entomology, University of Arizona, Forbes 410, 1140 E. South Campus Drive, Tucson AZ, 85721. Email: spstock@ag.arizona.edu

The field of entomopathogenic nematology has experienced exponential growth over the past decade. Recent years have seen an increased interest in studying entomopathogenic nematodes (EPN) not only because of their biological control potential, but also to answer other research questions in the fields of ecology, biodiversity, evolution, biochemistry, symbiosis and molecular genetics. Motivated by the escalating interest to search for fresh genetic material, hundreds of new isolates have and are currently been recovered worldwide. As a result, many new species and hundreds of new strains have been and are at present being described. Accurate identification of these insect parasites and pathogens is of critical importance. For example, matching the right nematode species with the appropriate target insect pest is relevant for the success of

biological control and integrated pest management (IPM) programs. For this purpose, molecular methods (i.e. sequencing of nucleic acids) have been widely accepted as quick diagnostic tool. One of the critical aspects to achieve accurate identifications of these nematodes is the establishment and maintenance of culture collections. EPN can be cultured both *in vivo* and *in vitro* considering a variety of methods and protocols. Limited collections of species/strain exist scattered in many laboratories worldwide. However, at present, a national and/or international depository for cultures does not exist. In this presentation I will review current methods considered for culturing of EPN. Moreover, I will discuss strategies for the creation and establishment of a 'stable' and 'secure' EPN collection to provide a resource for reference and for future use in biocontrol programs.

A-19

In Vitro Culture of an Entomopoxvirus from a Parasitic Wasp and Its Potential to Control Tephritid Fruit Flies. PAULINE O. LAWRENCE. Department of Entomology and Nematology, University of Florida, Gainesville, FL. Email: pol@ifas.ufl.edu

Viruses are potential biocontrol agents against insect pests but their effective use in control programs has been hampered, at least in part, by the lack of information on their genomes, taxon specificity, and their rate of post-infection pathogenicity. An essential tool in addressing these issues, is the development of insect cell lines that support virus replication and facilitate studies of viral gene expression and impact on host cells. Few cyclorhaphous dipteran viruses have been described and fewer still (or none) are cultured in dipteran cell lines. Our research focuses on a unique entomopoxvirus (EPV) that replicates in the accessory gland of the parasitic wasp *Diachasmimorpha longicaudata* (DI) (Braconidae), a biocontrol agent of *Ceratitis capitata* and *Anastrepha suspensa*, Old and New World fruit flies (Diptera: Tephritidae) respectively, and related species. The enveloped virion of DIEPV is 250-300 nm in diameter and contains a 250-300 kb unipartite, highly A-T (>60%) rich, linear DNA genome. DIEPV appears to be non-pathogenic to the wasp but destroys hemocytes of the fruit fly larval host and disrupts its cellular defense responses. Thus, DIEPV has the potential as a biocontrol agent, independent of the wasp vector. We developed the first *A. suspensa* embryonic cell line and evaluated embryonic cell lines from *C. capitata* and *Drosophila* (Kc) as well as an *l(2)mbn* tumorous blood (hemocyte) cell line from *Drosophila*, for DIEPV culture. The virus infects all three embryonic cell lines and replicates to a low level (<20% of cells) only in the

tephritid cell lines. It also replicates in ~35% of *l(2)mbn* cells, producing extra-cellular enveloped virus (EEV) similar to that seen in *in vivo* infections of *A. suspensa* larval hemocytes. We continue to improve the culture conditions for DIEPV replication to achieve at least 50% infection. This will facilitate fundamental studies of DIEPV gene expression and impact on host cells and provide insights into the potential of this unique virus in some aspect of tephritid fruit fly control.

A-20

Foreign Exploration of Potential Entomopathogen Agents for the Control of the Diamondback Moth, *Plutella xylostella*: a Case Study. JAMES J. GRASELA. USDA/ARS/BCIRL, 1503 S. Providence Rd., Research Park, Columbia, MO 65203-3535. Email: graselajj@missouri.edu

Participants from the USDA, ARS, Biological Control of Insects Research Laboratory (BCIRL), Columbia, Missouri and the Kenya Agricultural Research Institute (KARI)/National Agricultural Research Centre (NARC), Nairobi, Kenya conducted a joint field survey for virus-infected lepidopteran larvae on important agricultural crops such as maize, millet, cotton, and crucifers among other crops which are predominantly grown in the western Rift Valley, central highlands, eastern, and coastal agricultural regions of Kenya. KARI provided some transportation, qualified technicians and facilities to assist in the field survey and laboratory rearing of sampled insects. In addition, scientists at the International Centre of Insect Physiology and Ecology (ICIPE) and extension personnel of KARI stationed at various field laboratories throughout the country also provided information and assistance as to possible field sites from which to sample target insects. Of the 25 field sites investigated 15 of these contained pest insects that were found in sufficient numbers to warrant further sampling for diseased larvae. Either the absence or low numbers (<5) of larvae found at the remaining sampled sites were the result of either insecticide applications or heavy rainfall (short-rainy season in Kenya at the time of survey) which can drown insect larvae, particularly the tiny diamondback moth larvae. Many more field locations could have been sampled if it were not for the poor conditions of the paved roads as well as the difficulty in procuring transportation. Despite some of these problems, a total of 37 possible diseased insects were returned to the USDA, ARS, BCIRL for further examination and analysis.

A-21

Baculoviruses as Effective Bio-control Agents of Forest Pests. GUIDO CAPUTO. Natural Resources Canada

Canadian Forest Service, 1219 Queen St. E., Sault Ste. Marie, ON P6A 2E5, CANADA. Email: gcaputo@nrcan.gc.ca

Canada is a nation of trees and as such, a nation to a variety of forest insects both native including the spruce budworm (*Choristoneura spp.*) the #1 defoliators of spruce forests in North America and non-native, or introduced invasive insects including the Asian longhorned beetle (*Anoplophora glabripennis*) and the emerald ash borer, (*Agrilus planipennis*). Alien species are more threatening having no natural predators or diseases here to limit their growth. The end result of an infestation is an overall poorer quality tree. Chemical pesticides are not an environmentally acceptable option. Baculoviruses' safety, specificity and efficacy can make them just as an effective bio-control for a variety of forest pests. Having a biphasic replication cycle they propagate themselves both horizontally from insect to insect and vertically from cell to cell within the host. This session will focus on predicting the occurrence of forest infestations, identifying the responsible pest and the role baculoviruses can play in a biocontrol program. In nature baculoviral infection begins when a healthy insect ingests naturally occurring virus particles from a dead larva leading to the production of more virus particles until the cells, and ultimately the insect, dies. Viral DNA is extracted and molecularly verified to its origin. Baculoviruses can be amplified in the laboratory both in vivo in large volumes on artificial diet and quarantined reared insects and in vitro in the more than 125 continuous cell lines developed in our laboratory. These cell lines representing approximately 20% of insect cell lines in existence have been developed from the eastern spruce budworm (*Choristoneura fumiferana*), western spruce budworm (*Choristoneura occidentalis*), forest tent caterpillar (*Malacosoma disstria*), tobacco hornworm (*Manduca sexta*), white-marked tussock moth (*Orgyia leucostigma*), red-headed pine sawfly (*Neodiprion lecontei*), the white pine weevil (*Pissodes strobi*) and, the tarnished plant bug (*Lygus lineolaris*). They represent five tissues of origin, namely neonate larvae, embryos, ovaries, hemocytes, and midgut and four Insect Orders namely Lepidoptera, Hymenoptera, Coleoptera and Hemiptera. Presently, our cell lines are being used by 41 researchers in 8 Canadian provinces, 36 researchers in 19 US states and 28 researchers in 10 countries worldwide. The efficacy of baculoviruses can be enhanced by the insertion and expression, under the control of late and very late gene promoters, of specific toxins (scorpion toxin, mite toxin, *B. thuringiensis* toxin), hormones, enzymes or other foreign gene products exhibiting insecticidal activity. Similarly, deleting genes not essential for virus replication can also increase its activity. Removal of the gene which prevents infected larvae from molting (UDP-glucosyltransferase)

allows the infected larvae to molt, stop feeding and eventual die. Any construction and use of genetically-engineered baculoviruses does require rigorous laboratory and field trial assessments, the approval of a variety of regulatory agencies and open discussions between the scientists and the public.

A-22

Using the PTS (Portable Test System): Hand-held Technology for Rapid Assessment of Microbial Contamination. NORMAN R. WAINWRIGHT. Charles River Laboratories, Inc., 1023 Wappoo Rd., Suite 43-B, Charleston, SC 29407. Email: norm.wainwright@crl.com

As many industries seek to supplement standard culture-based techniques with more rapid, point-of-use assays for microbial evaluation, standards for procedures and data analysis must be established. The current standard in microbiological contamination studies is the colony forming unit (CFU), having been described and used as a means of quantification since solid bacterial growth media was introduced in the nineteenth century. Solid media provided microbiologists and other researchers with a simple method for culturing and quantifying microbes in a sample. Disadvantages exist in the use of solid culture media, including the time required before colonies can be counted (24-72 hrs.) and the fact that as many as 90% of bacterial species will not grow on available agars. Recently, alternate methods of detecting microbial contamination have been described, including the Limulus Amebocyte Lysate (LAL) assay. As the LAL assay is increasingly utilized in research and industry, a corresponding interest exists in attempting to relate the results from this test to other conventional tests. Results from the PTS will be shown from traditional endotoxin testing, protein concentration, gram type identification and surface contamination in the Pharmaceutical, Biotech and Aerospace industries.

A-23

Cell Line ID and Contamination Detection by Allele-specific Real-time TaqMan PCR Assays. Ruoying Tan and CAIFU CHEN. Genomic Assays R&D, Life Technologies, 850 Lincoln Centre Drive, Foster City, CA 94404. Email: chenx@appliedbiosystems.com

Novel allele-specific real-time TaqMan assay has been developed for detecting mutant SNP alleles in a mixed sample population. We demonstrate that it could quantitatively detect rare alleles with specificity of > 99.9%. Potential applications of this new assay includes rare mutation detection, allelic gene expression analysis, allelic DNA copy number analysis, human identification and cell line QC. We

have designed and tested a set of 48 allele-specific TaqMan SNP assays for cell line ID and contamination detection. Results demonstrate that these assays could not only identify cell line identity but also detect less than 0.1% contamination of cells from other cell lines.

A-24

Mycoplasmal Contamination: Risk Reduction Strategies and Diagnostic Methods. JILL MARIANO. Bionique Testing Laboratories, 156 Fay Brook Drive, Saranac Lake, NY 12983. Email: jamariano@bionique.com

Mycoplasma is considered to be one of the most prevalent and serious microbial cell culture contaminants in both research and industrial cell culture facilities. Reported rates of contamination have been in the range of 11-35% within established cell lines. Chronically infected cultures often occur with mycoplasmal contamination, since it often evades macroscopic and microscopic detection despite reaching concentrations of 10^7 – 10^8 cfu/mL in cell lines. Today, we have gained enormous recognition of the impact

of contamination on the host cell. The potential sequelae of infection include chromosomal alterations and aberrations in every aspect of cellular metabolism. Consequently, the verification of mycoplasma-free cell lines is essential in the forward process decision-making steps within research and biopharmaceutical manufacturing. Current FDA approved testing methods require a minimum of 28 d to complete. However, significant progress has been made in the development and validation of rapid microbiological methods for the detection of mycoplasma. The potential impact of these new analytical methods in the testing of cell lines and cell-derived products will be reviewed in this presentation. Additionally, strategies to reduce the risk of contamination and transmission of infection to uninfected cell lines by applying stringent quality control measures will be discussed. In summary, the consequences of failing to establish a robust mycoplasmal testing program in any cell culture laboratory can result in the compromise of research endeavors, financial losses and more importantly, pose potential biosafety concerns in those facilities involved with preclinical safety testing or biopharmaceutical manufacturing.