

Joint Symposia

J-1

Storage and Distribution Issues for Cryopreserved Cells and Tissues. KELVIN G. M. BROCKBANK^{1,2}. ¹Cell & Tissue Systems, Inc., North Charleston, SC and ²Visiting Faculty, Georgia Tech/Emory Center for the Engineering of Living Tissues, Georgia Institute of Technology, Atlanta, GA. Email: kbrockbank@celltissuesystems.com

The objective of this presentation is to review options for storage and distribution of mammalian cells and tissues. The two main cryopreservation strategies employ either traditional freezing or ice-free vitrification methods. Both approaches involve the application of fundamental cryobiology principles. Simply cooling cells or tissues with spontaneous ice nucleation and crystal growth usually results in dead, nonfunctional materials. The discovery of the benefits of glycerol in 1949 and shortly thereafter of dimethyl sulfoxide as cryoprotective agents, established the field of cryobiology and many other cryoprotective agents have since been identified. Often the requirements for cryopreservation of multicellular tissues are more complex than isolated cells. Adult stem cells and many established cell lines are easy to preserve using slow rate cooling freezing methods. However, some cell systems may be very difficult to cryopreserve, such as hepatocytes and platelets. Frozen tissues have extensive extracellular, interstitial ice formation following traditional cryopreservation procedures. Such frozen specimens may have excellent cell viability. However, in some cases the cells in the tissue may no longer operate as functional units. The extent of freezing damage depends upon the amount of free water in the system and the ability of that water to crystallize during cooling. In such situations ice-free vitrification strategies often provide the best outcome. Vitrification is the amorphous solidification of a supercooled liquid that can be achieved by adjusting the solute composition and the cooling rate such that nucleation and growth of ice crystals is essentially prevented. Vitrification has been applied successfully to the storage of cells and tissues including human embryos with excellent pregnancy outcomes. Other factors, in addition to ice formation, that have biological consequences during preservation will be discussed and

last, but by no means least, temperature control during storage and transport of cryopreserved specimens.

J-2

Preservation of Biomaterials in the Dry State: Lessons from Nature. JOHN H. CROWE. Department of Molecular and Cellular Biology, University of California, Davis, CA 95618. Email: jhcrowe@ucdavis.edu

Trehalose is a sugar found at high concentrations in organisms that are capable of entering a dry state, known as anhydrobiosis. This sugar has the capacity to preserve biological materials such as membranes, proteins, and nucleic acids in such a dry state. Several commercial products have come into use as a result of these findings. Subsequently, a myth has grown up about the properties of trehalose. In this talk I will show what is true about trehalose and what is not. This sugar does have unusual physical properties that make it useful, but it is not the magic bullet that some sources would suggest. Then I will show that this sugar may be useful in preserving dry mammalian cells, including platelets, red blood cells, and nucleated cells. Finally, some recent data on preservation of nucleic acids are leading to additional commercial products. (Supported by grants from NIH and DARPA).

J-3

Beyond the Cell Wall: a Comparison Between Plant and Animal Cell Cryopreservation. HUGH W. PRITCHARD¹ and Barry J. Fuller². ¹Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK and ²University Department of Surgery and Liver Transplant Unit, Royal Free Hospital and UCL School of Medicine, London NW3 2QG, UK. Email: h.pritchard@kew.org, b.fuller@medsch.ucl.ac.uk

Fifty years on from the introduction of the use of cryoprotectants, cell/tissue banking by cryopreservation has become routine in many areas of biotechnology, medicine, plant breeding and conservation. Often empirical studies are required for success; however, for plant cell/tissues, cryopres-

ervation methodologies have approached the generic, with encapsulation/dehydration and vitrification solution-based techniques applicable to hundreds of species. Whilst mixtures of cryoprotective agents (CPA) are devised to enable the formation of low temperature glasses, in seeds that are naturally desiccation tolerant, drying at warmer temperature induces glass formation thus generally circumventing consideration of subsequent cooling/warming rate control. Such extremophily in seeds is not universal, as some seeds are sensitive to drying. One consequence of desiccation stress in seeds is the initiation of programmed cell death modulated by changes to glutathione, a major cellular antioxidant and redox buffer, in a way similar to that observed in human cells. Similarly, antioxidant defences are needed for the successful recovery of normal metabolism after cold preservation of organs. Another group of seeds shows sensitivity to dry, cold storage, often in the range -50 to 5°C . A close association between viability loss and structural changes in seed oils appears similar to proposed mechanisms for pig embryo sensitivity to cryoconservation. Tissue oil content also has a profound effect on cellular unfrozen water content in both plant and animal cells. Such convergences in the successes and challenges in plant and animal cell cryopreservation indicate that much can be learned from looking beyond the cell wall.

J-4

Design, Optimization and Handling of Mammalian Cell Culture Media. PAUL J. PRICE. D-Finitive Cell Technologies, 1023 Wappoo Rd, Suite 33-B, Charleston, SC 29407. Email: p.price05@comcast.net

The cell culture medium is a dynamic mixture consisting of amino acids, vitamins, a source of energy, growth factors, trace minerals and other components in a buffered salt solution. Each component has a shelf life, sensitivity to the physical environment and a variety of interactive and breakdown products. The Classical mammalian cell culture formulations require further supplementation with a protein source such as serum and were designed using cancer-derived cell lines. These Classical formulations for mammalian cells can be very sub-optimal for the growth of specialized cells, such as stem cells and differentiated cells. This seminar will start with the role and problems associated with some of the key basic components of the medium and then show how Industry has been able to progress from serum-requiring to serum-free, to animal-component-free and then to chemically-defined formulations. Emphasis will be placed on how the cell culturist can reduce apoptosis by controlling the physical environment and the production of ammonia and free radicals through the optimization of the media formulation and the proper handling of the cells.

J-5

A Systematic Approach to Media Development with an Emphasis on Optimization at the Basal Component Level. SOVERIN KARMIOL and Matt V. Caple. SAFC Biosciences, Cell Sciences and Development, 2909 Laclede Ave., St. Louis, MO 63103. Email: soverin.karmiol@sial.com

A formal definition of medium optimization is possible based on the observation that each factor influencing the endpoint can be characterized by a dose response curve. The optimized state is achieved when all the factors are at their peak level. This distinguishes medium optimization as systematic process rather than a hunt for the appropriate media components and their concentrations. The practice of hunting for media components is characterized by a trial and error approach resulting in more components than is necessary. An emphasis on the optimization of the basal components is warranted since even in the presence of optimal concentrations of signaling molecules no assurance of appropriate performance can be guaranteed if essential component concentrations are either deficient or inhibitory. Due to the interactive nature of media components experiments guided by a DOE strategy is most fruitful. Also, DOE is an efficient experimental approach reducing development time. Further, since the number of factors, the majority of which are media components, are large an investigation into the appropriate levels of these factors requires HTS to manage them and the resulting complexity. Data will be presented to support these statements using CHO cells.

J-6

Medium Changes During the Culture Cycle Influence Tobacco Suspension Cell Physiology. JEAN ROBERTS, Erika Snodderley, Anthony Snyder, Rob Peterson, Karl Schnelle, and Robbi Garrison. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jlroberts2@dow.com

The composition of the medium undergoes striking changes during a weekly culture cycle for tobacco cells under batch cultivation. By following changes in carbon nutrient availability, osmolarity, conductivity, and pH as the cells multiply and consume nutrients, we can formulate hypotheses about changes in underlying cell metabolism and physiology. We can also identify sources of physiological stress arising from these medium changes. Transition from carbon sufficiency to autophagy can be observed during entry to stationary phase. Subsequent subculture into fresh medium results in cells responding to hyperosmotic stress with medium acidification. These changes in the culture medium and accompanying cell metabolism can be infor-

mative as we use the cells for transformation, protein expression, or other experimental uses.

J-7

Investigation of the Metabolism of *Nicotiana tabacum* Cells Using the Respiratory Monitoring System (RAMOS). DAVID A. ULLISCH¹, Jean L. Roberts², W. Treffenfeldt², and Jochen Büchs¹. ¹Chair of Biochemical Engineering, RWTH Aachen University, Aachen, GERMANY and ²Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: david.ullisch@avt.rwth-aachen.de

For the development of biotechnological processes the composition of the medium is of essential importance. A commercially available medium for plant cell culture merely consists of mineral nutrients, growth hormones and a carbon source, for instance glucose or sucrose. Therefore, the growth of plant cells in suspension cultures is characterized through a simple medium composition and an easy handling. However, these commercially available media are based on traditional recipes and offer quite a huge potential for optimization, because these media are not optimized to the specific growth and productivity of a certain cell line. An optimal composition of the medium is vitally important, since an ideal medium improves growth and production of the target protein of plant cells. In this study, the influence of the nutrients and the extra addition of nutrients during the cultivation on the protein expression in plant cells are investigated. For such a media characterization online monitoring gives ideal insight into the physiological state of the cell. For this purpose we used the Respiration Activity Monitoring System (RAMOS) for online measurements of the respiration activity parameters in shake flasks, oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and the respiratory quotient (RQ), described by Anderlei and Büchs 2001 [1] and Anderlei et al. 2004 [2]. Measuring the OTR online during cultivation is a suitable way to characterize the physiological state of the cells since most metabolic activities are connected to oxygen consumption. For example, nutrient or oxygen limitations, diauxic growth and product inhibition could be identified. In this study we investigate the changes in nutrient composition in shake flask cultures over cultivation time. We demonstrate that changes in the medium have significant influence on the metabolism of the cell. By monitoring the respiration activities of the plant cells a diauxic growth and a limitation by a second substrate apart from the carbon source could be detected.

1. Anderlei, T. and Büchs, J., Device for sterile online measurement of the oxygen transfer rate in shaking flasks. *Biochemical Engineering Journal*, 2001. 7(2), 157–162

2. Anderlei, T.; Zang W.; Paspasyrou, M. and Büchs, J., Online respiration activity measurement (OTR, CTR, RQ)

in shake flasks. *Biochemical Engineering Journal*, 2004. 17 (3), 187–194

J-8

Use of Multiple In Vitro Assays to Study Pleiotropic Anti-inflammatory and Anti-metastatic Actions of Low Molecular Weight Natural Products of Botanical Origin. S. R. SIMON, S. Zamurrad, K. Fenwick, S. Parrino, F. Daccueil, and E. J. Roemer. Pathology 8691, SUNY at Stony Brook, NY 11794. Email: ssimon@notes.cc.sunysb.edu

Natural products from botanical sources have been appreciated for their bioactive constituents, but they continue to provide new insights into basic regulatory mechanisms in cells and tissues. While their modulation of individual cell functions or enzymes may be modest compared to the high potency of synthetic pharmaceutical agents, the natural products are often capable of achieving a safer outcome because of their pleiotropic modes of action. Their reduced toxicity may be attributed to their capacity to inhibit or activate each of their multiple targets in the cell only partially, so that endogenous mechanisms of homeostasis can take over and restore normal function. An example of the pleiotropic activities of botanical constituents may be seen in the multiple modes of action of gallic acid derivatives, as found in green tea catechins and components of clove bud oil. These compounds have been recognized as antioxidants, but more recently they have been shown to have additional capacity to inhibit the activity of zinc metalloproteinases and even the serine proteinase neutrophil elastase. Inhibition of each targeted enzyme is not total, but the combined effects interrupt the synergy between destructive proteinases that probably contributes to the anti-inflammatory action of the natural products with minimal toxicity. We are now studying their interactions with other zinc metalloenzymes such as histone deacetylases implicated in ageing and neoplasia. The complex actions of compounds such as the catechins and the gallates that modulate steps in cell regulation to achieve therapeutic effects cannot always be studied by classical determinations of enzyme kinetic parameters, but are more amenable to investigation using organotypic human cell-based models.

J-9

Anti-tumor Mechanisms of Various *Scutellaria* Extracts and Constituent Flavonoids. P. PARAJULI¹, N. Joshee², A. Rimando³, S. Mittal¹, and A. K. Yadav². ¹Department of Neurosurgery, Wayne State University & Karmanos Cancer Institute, Detroit, MI 48085; ²Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030; and ³USDA-ARS, University Ave, University, MS 38677. Email: pparajuli@med.wayne.edu

Plants of genus *Scutellaria* constitute a common component of Eastern as well as traditional American medicine against various human ailments, including cancer. We have recently reported a comprehensive analysis of the leaf, stem and root extracts obtained from thirteen different species of *Scutellaria* for their flavonoid content as well as for their mechanism of anti-cancer activity. Among the thirteen species examined, the leaf extracts of *S. angulosa* (*SanL*), *S. integrifolia* (*SinL*), *S. ocmulgee* (*SocL*) and *S. scandens* (*SscL*) showed consistent, dose-dependent anti-proliferative and pro-apoptotic activities against various malignant cell lines. *Scutellaria* extracts as well as the flavonoids significantly inhibited in vitro growth of malignant brain (U87-MG glioma) and breast cancer (MDA-MB-231) cells without affecting the growth of corresponding non-malignant (NHA and HMEC) cells. These results showed that *Scutellaria* extracts or isolated flavonoids target molecular mechanisms that are specific to the malignant phenotype. The PI3K/Akt signaling pathway is arguably the major pathway that regulates cell proliferation and apoptosis in malignant tumors, including gliomas. The signaling molecule Akt/PKB is constitutively phosphorylated in majority of gliomas. We observed significant inhibition of Akt phosphorylation in glioma cells by *Scutellaria* extract in a dose- and time-dependent manner. In an in vitro kinase assay, *Scutellaria* extract and wogonin also inhibited phosphorylation of GSK by Akt, suggesting that active components in *Scutellaria* could directly bind to Akt and hinder its kinase activity. Immunohistochemical analysis of in vivo transplanted gliomas also revealed significantly reduced Akt activity following administration of *Scutellaria*. Overall, these results suggested that active phytochemicals in *Scutellaria* directly or indirectly target PI3/Akt signaling pathway for its anti-tumor activity.

J-10

Plant Extracts for Cosmeceutical Applications: Genetic Profiling of Cell Responses. M. MONAGHAN. Center for Biotechnology, Stony Brook University, Stony Brook, New York. Email: Melissa.monaghan@stonybrook.edu

With the success of the Human Genome Project, the scientific community has access to the sequence information for the human genome. In combination with technological advances, researchers have the ability to fabricate DNA microarrays to print tens of thousands of these genes onto a glass slide. Microarrays investigate beyond a handful of targets and toward a global snapshot of the cells transcriptional activity. In our study, normal human dermal fibroblasts (NHDF) will be cultured in the presence or absence of ascorbic acid, plant extracts and other skin care ingredients. RNA samples, as small as 100 nanograms, are reverse transcribed into fluorescently labeled cDNA and hybridized to human microarrays. The gene expression data is filtered by statistical tests and by fold change to identify significant genetic changes in the dermal fibroblasts. For cosmeceutical applications, we focus on genes routinely regulated in the extracellular matrix, anti-inflammatory responses, and for pro-pigmentation proteins. The additional benefit of microarrays is the ability to interrogate broad classes of genes. The experiment does not limit the data to skin applications but open opportunities to discover novel uses. It provides the researcher direction in testing the functionality of the plant extracts. Microarrays are a powerful tool to discover various applications for plant extracts. By studying the genetic profiles generated by the different treatments on the NHDF, we can identify potential cosmeceutical targets and also suggest new applications. Societies across the globe have cultured plants for traditional uses. As modern studies find new uses for traditional plant extracts, its uses will increase worldwide.