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Plenary Symposia

PS-1

Quantifying Heterogeneity in Plant Cell Culture Using Flow Cytometric Methods. SUSAN C. ROBERTS, Worcester Polytechnic Institute, Department of Chemical Engineering, 100 Institute Road, Worcester, MA 01609. Email: scroberts@wpi.edu

Plants synthesize a cohort of specialized metabolites in response to stressors that enable plant survival and propagation in the wild. These specialized metabolites are sophisticated chemical structures that are often useful not only to the native plant as part of the defense response.Plant cell culture is an alternative production technology for complex natural products that cannot be chemically synthesized or extracted in high yields from natural sources. Suspension cultures consisting of dedifferentiated plant cells can be grown in liquid media *in vitro*, and are easily scalable using traditional industrial bioprocess technology. However, in comparison with microbial or mammalian systems, plant cell culture presents distinct challenges, including aggregation in culture, low and variable product yields and slow growth rates. One of the primary goals of our research is to understand culture heterogeneity and specifically cell-cell variation in metabolism in order to design superior bioprocesses for synthesis of natural products. Our primary system of study is *Taxus* suspension cultures for production of the anticancer agent paclitaxel (Taxol™). In this talk, we will discuss the development and application of flow cytometric methods to understand variations in cellular metabolism in *Taxus*. We have developed methods to isolate individual particles (e.g., cells, protoplasts, nuclei) from *Taxus* suspension cultures and using flow cytometry have identified specialized cell sub-populations with characteristic growth and paclitaxel production patterns. In our work, we have adapted a variety of stains for their use in plant suspension cultures to understand viability (apoptosis vs. necrosis), cell cycle participation and protein production. The approaches we have established can be easily translated to other plant systems of study to gain information on cellular heterogeneity.

PS-2

Sterile Sorting of Human Cells for In Vitro Manipulation and Expansion Prior to Clinical Transplantation. K. BECK, E. Rodriguez-Mesa, M. Ragland, and J. Dunne. Miltenyi Biotec Inc., Auburn, CA. Email: kevinb@miltenyibiotec.com

Flow cytometric sorting technology was invented over 50 years ago. While aspects of the technology have seen iterative improvements, the current methods remain fundamentally unchanged. Flow sorters generally rely on electrostatic jet-in-air mechanisms where cells are passed under high pressure through an oscillating nozzle to create droplets. Droplets are then electrically charged and guided towards collection vessels as they pass charged deflection plates. The MACSQuant Tyto represents a paradigm shift where high-speed mechanical sorting is facilitated by the world's fastest valve, which works to divert the path of cells as they travel through a microfluidic channel, ultimately assorting them into their collection chambers. The novel design allows for gentle, low-pressure sorting that requires minimal operator expertise. Sorting is conducted in a closed sterile cartridge, enabling cell sorting without concerns about sterility or biosafety. Accordingly, the Tyto provides a simple method of multi-parameter cell purification for both clinical and research applications. MACSQuant Tyto applications will be discussed including sorting of cancer-specific cytotoxic lymphocytes for clinical transplantation, purification and subsequent expansion of mesenchymal stem cells for use in treatment of diabetic ulcers and isolation of extremely rare circulating tumor cells.

PS-3

The Path to Product: Solving Agriculture's Greatest Challenges One Microbe at a Time. CHAD A. KEYSER and the AgBiome Team. AgBiome, Research Triangle Park, NC 27703. Email: ckeyseragbiome.com

Agricultural crops face continual pressure from both pests and diseases. Despite living in the most technologically advanced

and innovative time in human history, no major new modes-of-action for protecting crops have been introduced to the market for many decades. AgBiome has assembled a highly experienced team with a long history of successfully delivering agbiotech products to market. This team is now harnessing the power of the plant and soil microbiome, and has established a platform that facilitates quick discovery and development of traits and biologicals for plant protection. This platform has resulted in the expected 2017 launch of our first biological product, Howler, which is efficacious against multiple fungal pathogens. Agbiome also has numerous trait and additional biological projects, which have seen significant advancements in the last year; these will provide solutions to many of the most important insect, nematode and disease pests in agriculture. In a project funded by the Bill and Melinda Gates Foundation, AgBiome is now employing its technology and experience to tackle some of the most devastating agricultural challenges in Sub-Saharan Africa. We are working to isolate, identify and develop new plant-associated microbes that can be used as biological insecticides and fungicides against key African pests, including sweetpotato weevil sorghum anthracnose, black Sigatoka, and aflatoxin-producing fungi. We have only begun to understand the far-reaching potential contained in the microbiome, but it is clear that microbes will play an important role in the future in crop protection.

PS-4

Current and Emerging Technologies in the Study of Beneficial Modulators of the Gut Microbiome. M. ANDREA AZCARATE-PERIL. Department of Medicine and Microbiome Core Facility, Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, Chapel Hill, NC. Email: andrea_azcarate-peril@med.unc.edu

Connections between gut microbiome modulation and host health have become evident and are currently a research target across disciplines. As an intrinsically multidisciplinary field, microbiome research has benefited from technological advancements in systems and synthetic biology, biomaterials engineering, and traditional microbiology. Moreover, the field has been revolutionized by high-throughput sequencing technologies, permitting compositional and functional analyses that were previously an unrealistic undertaking. Newly emerging technologies including engineered organoids derived from stem cells, high-throughput culturing, and microfluidics assays are producing novel approaches and protocols to improve the efficiency and quality of microbiome research. This presentation will review emerging technologies and their application to comprehend beneficial modulation of the gut microbiome by prebiotics and probiotics.

PS-5

Packed-bed Reactors, Dynamic Culturing, and Stress-Directed Microbiome Selection; a Case Study of Agrochemicals Soil Biodegradation. JOSE M. BRUNO-BARCENA. North Carolina State University, Department of Plant and Microbial Biology, College of Agriculture and Life Sciences, Raleigh, NC. Email: jbbarcena@ncsu.edu

Improvements of packed-bed reactors over the past two decades have focused on system stability through reactor design, support materials, and operational conditions. Altogether these stable, dynamically operated reactors display high similarities with the natural world including matrices with high surface/area ratios, high biomass retention of biofilm forming and planktonic communities, and distinct community resistance to toxicity. Since these operational systems are good tools for studying natural biological processes, we performed dynamic stress-directed in-vitro community selection. This allowed for the retention and enrichment of the metabolically relevant agrochemical- exposed soil microbiome. After exposing to the same agrochemical, diversity variations of the stable reactor communities and the initial challenged soil samples were compared providing mutual data validation. We will discuss some of the major benefits, among many, of the development of this technology including the consistent and reproducible generation of biomass and metabolites of interest.

PS-6

Plantimals: Plant Tissues as Scaffolds for Human Tissue Engineering. J. Gershlak[1], S. Hernandez[2], G. Fontana[3], L. Perreault[1], K. Hansen[1], S. Larson[2], B. Binder[4], D. Dolivo[2], T. Yang[5,6], T. Dominko[2,7], M. Rolle[1], P. Weathers[2], F. Medina-Bolivar[5,6], C. Cramer[5,6], W. Murphy[8,9], and G. GAUDETTE[1]. [1]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA; [2]Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA; [3]Orthopedics and Rehabilitation, University of Wisconsin School of Medicine and Public Health, Madison, WI; [4]Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI; [5]Department of Biological Sciences, Arkansas State University, State University, AR; [6]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR; [7]Center for Biomedical Sciences and Engineering, University of Nova Gorica, Slovenia; [8]Biomedical Engineering, University of Wisconsin-Madison, Madison, WI;

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Despite significant advances in tissue engineering, perfusion of thick (>300 μm) tissue remains a major challenge largely due to the difficulties associated with creating a viable perfusion network. Decellularization of mammalian tissue leaves a scaffold with a perfusion network, but the expense and availability of these tissues has limited its application. However, plant tissues are readily available and come in many different shapes and sizes. By taking advantage of the vascular structure of plant tissues, we developed decellularized plant tissue as a pefusable scaffold for tissue engineering applications. Perfusion-based decellularization can be modified for different plant species, providing a wide variety of geometries as a basis for tissue engineered structures. To demonstrate the patent vasculature, microparticles where perfused in decellularized leaf scaffolds. Microparticles with a diameter of 10 μm were able to transverse the vasculature whereas 50 and 100 μm diameter microparticles were mostly trapped in the leaf scaffolds. To demonstrate the potential of decellularized plants to serve as a scaffold for engineered tissue, human pluripotent stem cell derived cardiomyocytes were seeded on the outer surfaces of plant scaffolds. Cardiomyocytes demonstrated contractile function and calcium handling capabilities over the course of 21 days. This work demonstrates the potential of decellularized plants to serve as a perfusable scaffold for tissue engineering. Decellularized plants may provide a cost-efficient, "green" technology for regenerating large volume vascularized tissue mass.

PS-8

Berry Anthocyanins and Metabolic Syndrome. J. Overall[1,2], S. A. Bonney[1,3], D. Esposito[1,3], and S. KOMARNYTSKY[1,2]. [1]Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus, 600 Laureate Way, Kannapolis, NC 28081; [2]Department of Food, Bioprocessing & Nutrition Sciences, North Carolina State University, 400 Dan Allen Drive, Raleigh, NC 27695; and [3]Department of Animal Science, NC State University, 120 Broughton Drive, Raleigh, NC 27695. Email: komarnytsky@ncsu. edu

Overconsumption of energy dense foods and sedentary lifestyle are considered as major causes of obesity-associated insulin resistance and abnormal glucose metabolism. Results from both cohort studies and randomized trials suggest that anthocyanins from berries may lower the metabolic risks, however these reports are equivocal. The present study was designed to examine effects of 6 berries with structurally diverse anthocyanin profiles (normalized to 400 μg/g total anthocyanin content) on development of metabolic risk factors in the C57BL/6 mouse model of polygenic obesity. Diets supplemented with blackberry (mono-glycosylated cyanidins), black raspberry (acylated mono-glycosylated cyanidins), blackcurrant (mono-and di-glycosylated cyanidins and delphinidins), maqui berry (di-glycosylated delphinidins), Concord grape (acylated mono-glycosylated delphinidins and petunidins), and blueberry (mono-glycosylated delphinidins, malvidins, and petunidins) showed a prominent discrepancy between biological activities of delphinidin/malvidin- versus cyanidin-type anthocyanins that could be explained by differences in their structure and metabolism in the gut. Consumption of berries also resulted in a strong shift in the gastrointestinal bacterial communities towards obligate anaerobes. Further work is needed to understand mechanisms that lead to nearly anoxic conditions in the gut lumens, including the relative contributions of host, diet and/or microbial oxidative activity, and their implication to human health.

PS-9

Dietary Bioactives for Tissue Regeneration and Skin Care. DEBORA ESPOSITO. North Carolina State University, Plants for Human Health Institute, 600 Laureate Way, Kannapolis, NC 28081 and Department of Animal Science, 120 Broughton Drive, Raleigh, NC 27695. Email: daesposi@ncsu.edu

The topical application of plant derived preparations to promote wound healing or skin regeneration is a common practice in many cultures, yet both the chemical characterization of the preparations and studies aimed at identifying the underlying mechanisms involved in the wound healing and tissue repair processes are surprisingly scarce. Dietary phytochemicals are known to exhibit a variety of anti-inflammatory and anti-microbial activities, which are the very desired properties for wound healing and tissue repair bioactive candidates. From Alaskan berries to Easter lilies, there are hundreds of remarkably common herbs, flowers, berries and plants that restore inflammatory and metabolic balances. Underlying mechanisms by which plant bioactives produce a therapeutic effect in fibroblast and keratinocyte cell cultures were determined. Cell migration assays offer the distinct advantage of not damaging the cells. Cell exclusion zone format also allows continuous visual assessment of the cells throughout the experiment with the ability to acquire multiplexed data. Information was collected regarding morphology, velocity, distance and direction of migrating or invading cells as well as additional phenotypic effects of test compounds. This research approach is a prelude to establishing potential cosmeceutical applications (skin care) using plants as a prime ingredient.