

Animal Contributed Papers

A-1000

Habitat Restoration and Production of Bioactive Compounds from 3D Sponge Cell Cultures. MEGAN CONKLING, Elizabeth Urban-Gedamke, Amy E. Wright, and Shirley A. Pomponi. Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946. Email: mconkli2@fau.edu

Sponges are ecologically, commercially, and biomedically important organisms, but wild harvest is not enough to meet the demand for research and development of sponge-derived bioproducts. A recent study demonstrated our ability to culture marine sponge cells in three-dimensions (3D) using Fibracel® disks, Ultra-Low Temperature Agarose (ULTA) thin hydrogel layers, and ULTA gel microdroplets (Urban-Gedamke *et al.* 2021). The creation of a 3D sponge from cryopreserved cells has potential applications for habitat restoration and the production of bioactive materials. Here we report the 3D cultures of three sponges that produce bioactive compounds (discodermolide from *Discodermia* spp., topsentins from *Spongosorites* spp., and stevensine from *Axinella corrugata*). We also report the 3D culture of habitat-building sponges (*Xestospongia muta* and *Geodia* cf. *gibberosa*) found on reefs and in seagrass environments. Cells were successfully cultured in an optimized nutrient medium and formed aggregates that attached to Fibracel® disk fibers as well as within ULTA hydrogel layers. We will discuss the advantages and disadvantages of each method, the production of bioactive compounds from these 3D cultures, and whether these 3D culture methods are a sustainable technique for *in vitro* production of marine natural products and habitat restoration. Ongoing research is focused on the scale-up of these 3D culture methods to produce marine sponge-derived natural products from other species and to establish nurseries for sponge restoration in habitats impacted by climate change, extreme weather events, or harmful algal blooms.

A-1001

Assessing the Apoptosis Effect of Prenylated Stilbenoids Combined with Paclitaxel in Triple-negative Breast

Cancer Cells. S. MOHAMMADHOSSEINPOUR^{1,2}, A. Weaver¹, L.-C. Ho¹, and F. Medina-Bolivar^{1,3}. ¹Molecular Biosciences Graduate Program, College of Sciences and Mathematics, Arkansas State University, Jonesboro, AR 72467; ²Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72467; and ³Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72467. Email: fmedinabolivar@astate.edu

Breast cancer is one of the most prevalent types of cancer in women worldwide. Triple-negative breast cancer (TNBC) is unresponsive to typical hormonal treatments causing it to be one of the deadliest forms of breast cancer. Investigating alternative therapies to increase survival rates for this disease is essential. This study aimed to examine if prenylated stilbenoids from peanut can act as an adjuvant for paclitaxel, a chemotherapeutic drug with severe side effects. The prenylated stilbenoids arachidin-1 (A-1) and arachidin-3 (A-3) are analogs of resveratrol (RES) and were produced in hairy root cultures of peanut. The cytotoxicity activity of A-1, A-3, and RES was studied in TNBC cell lines MDA-MB-231 and MDA-MB-436. Furthermore, the cytotoxicity of A-1, the most potent prenylated stilbenoid, combined with paclitaxel was studied by checkerboard assays in the TNBC cell lines. The apoptotic effects of this combination treatment were studied by western blotting targeting protein expression levels of PARP, caspase-8, caspase-9, and survivin and through the Apo-ONE Homogeneous Caspase-3/7 assay. To further investigate the apoptosis and cell cycle stages, cells treated with prenylated stilbenoids or RES were studied using flow cytometry. After 24 hours of treatment, A-1 exhibited higher cytotoxicity than A-3 and RES with approximately 11-fold and 6-fold lower IC₅₀, respectively, in MDA-MB-231 cells, and 9-fold and 8-fold lower IC₅₀, respectively, in MDA-MB-436 cells. A-1 did not show significant cytotoxicity in the non-cancerous cell line MCF-10A. Cytotoxicity, checkerboard, and flow cytometry assays showed a decrease in paclitaxel concentration when combined with prenylated stilbenoids. This highlights the significance of continuing research with prenylated stilbenoids as an adjuvant in TNBC treatment.

A-1002

A Flavonoid-rich Extract of *Moringa oleifera* (Morinaceae) Leaf Cultivated in Brazil Inhibited Inflammatory Mediators in Lipopolysaccharide-treated Macrophages. LARISSA SILVA^{1,2,3}, Jade Schlam^{1,2}, Leandro Ferreira³, Silvana Zucolotto^{1,2,3}, and Debora Esposito^{1,2}. ¹Plants for Human Health Institute, North Carolina State University, NCRC, Kannapolis, NC; ²Department of Animal Science, NCSU, Raleigh, NC; and ³Federal University of Rio Grande do Norte, Natal, BRAZIL. Email: lpeireir2@ncsu.edu, daesposi@ncsu.edu

Moringa oleifera is a globally recognized herbal medicine due to its pharmacological and nutritional properties, which correlate to the high contents of flavonoids in their leaves. Various persistence diseases are closely associated with excessive inflammation and/or consistent presence of radical oxygen species. Exposure of mammalian cells to LPS leads to the release of pro-inflammatory cytokines and, in turn, activates inflammatory cascades via the secretion of cytokines, lipid mediators, and adhesion molecules, that are common genetic biomarkers involved in the LPS-stimulated murine RAW 264.7 macrophage model inflammatory response *in vitro*. This present study aimed to evaluate the anti-inflammatory activities of an optimized flavonoid-rich hydroethanolic extract of *M. oleifera* leaf (MoHLE) and determine the influence of soil cultivation conditions (named T0, T1, T2 and T3) and harvest season of *M. oleifera* leaf from Northeast of Brazil, in provided anti-inflammatory activities on reactive oxygen species (ROS) and nitric oxide (NO), and inflammatory cytokines in LPS-treated RAW 267.4 cells. As a result the major compounds were characterized by HPLC-ESI-QTRAP-MS/MS in as glycosidic forms derived from apigenin, quercetin, and kaempferol. MoHLE exhibited safety and suppression of generation of nitric oxide (NO) and reactive oxygen species (ROS) between 5 and 50 µg/mL. And down-regulated the transcriptional levels of inflammatory regulatory genes including IL-4, IL-6, IL-1β, COX-2 and iNOS. These findings results showed the potential of this extract as a anti-inflammatory agent to develop new pharmaceutical products, and will contribute to the quality control and standardization of raw materials from *M. oleifera*, and As far as we know, this is the first attempt to grow open field cultivation of *M. oleifera* leaves in Brazil.

A-1003

Successful Scale Up of Marine Sponge Cell Cultures in 3D Bioprinted Hydrogel Microdroplets. ELIZABETH URBAN-GEDAMKE, Megan Conkling, and Shirley A. Pomponi. Harbor Branch Oceanographic Institute, Florida Atlantic

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Sponges are the most prolific source of marine natural products, but clinical development of these biomedically relevant compounds cannot be sustained by wild harvest or aquaculture. *In vitro* cell culture has been proposed as an alternative. For decades, marine sponge cell culture has been stymied by an inability to obtain cell lines. Recent developments in marine sponge cell culture have resulted in rapidly dividing sponge cells (Conkling *et al.* 2019) that can be immobilized in hydrogel microdroplets (GMDs) (Urban-Gedamke *et al.* 2021), although this 3-D microdroplet method was labor intensive and produced a low yield of GMD's. Here we report an improved method for generating larger quantities of GMD's using a 3-D bioprinter (BioX2, Cellink). We have produced GMD's containing sponge cells that metabolize nutrient media, undergo rapid cell division, and can be maintained long-term. These methods can be applied to 3-D cell cultures of sponge species that produce marine natural products to meet preclinical and clinical supply needs. With further optimization, more complex 3-D printed hydrogels can be constructed to mimic the internal architecture of a marine sponge and may be advantageous in the study of sponge cell interactions and differentiation.

A-1004

Antimicrobial Studies of 1,3-Diphenylpyrazole-derived Anilines Against Methicillin-resistant *Staphylococcus aureus*. HANSA RAJ KC^{1,3}, David Gilmore², and Mohammad A. Alam³. ¹Molecular Biosciences Program, ²Dept. of Biological Sciences, and ³Dept. of Chemistry and Physics, Arkansas State University, Jonesboro, AR. Email: hansa.kc@asmil.astate.edu

Several bacteria (the "ESKAPE" pathogens) are associated with nosocomial infections and antimicrobial resistance, including *Staphylococcus aureus*, is considered a high threat to human health. The development of new antibiotics is one of the strategies recommended by the CDC to combat the increased prevalence of antibiotic-resistant bacterial infections. Following compound synthesis, compounds were screened for antimicrobial activity using minimum inhibitory concentration testing (MIC) against various strains of *S. aureus* including methicillin-resistant *S. aureus* (MRSA). Compounds with low MICs were further investigated to determine their Minimum Bactericidal Concentration (MBC), ability to inhibit biofilm formation and destruction of preformed biofilms *in vitro*, time-kill assay to determine their bacteriostatic or bactericidal properties, persister kill assay to determine their ability to eliminate

MRSA persists, and multistep resistance to determine resistance accumulation. MIC values as low as 0.78 $\mu\text{g}/\text{mL}$ were observed among the compounds tested. For additional testing, compounds with MICs ranging from 6.25 $\mu\text{g}/\text{mL}$ to 0.78 $\mu\text{g}/\text{mL}$ were selected. MBC test eliminated bacteria at as low as 3.125 $\mu\text{g}/\text{mL}$ concentration. At 2 \times MIC concentrations, biofilm inhibition was observed almost 100% and pre-formed biofilm removal was around 90%. Three of the compounds were found to be bactericidal and one bacteriostatic against MRSA in a time-kill assay. MRSA persisters were significantly reduced over the four-hour treatment period at 8 \times MIC in comparison to the untreated and positive controls. The preliminary result of multistep resistant testing showed a four-fold increase in initial MIC value in the course of 14 passages. In this presentation, we will discuss the above antimicrobial properties of the 1,3-diphenylpyrazole derivatives along with on-going *in vivo* antimicrobial studies to clear bacterial infection in *Galleria mellonella*.

A-1005

Moderate Heat Assisted Electrotransfer as an Effective Means for Delivering Molecules to Cells and Tissue. RICHARD HELLER, Jody Synowiec, Samantha Mannarino, Julie Singh, and Guilan Shi. Department of Medical Engineering, University of South Florida, Tampa, FL. Email: rheller@usf.edu

Electrical-based therapies have been utilized successfully for the past three decades. A major focus area has been the treatment of solid tumors. This approach has demonstrated great potential as an ablative technique (irreversible

electroporation; IRE) as well as for drug (electrochemotherapy; ECT) and gene (gene electrotransfer; GET) delivery. While these have been significant achievements, the technology has not had any major advances in the past couple of decades and still falls short in achieving general acceptance as an effective therapeutic approach. Our research team has been exploring the use of electrical-based therapies, particularly GET and has established several protocols to several tissue types and for a variety of potential therapeutic applications including immunotherapy for cancer, delivery of DNA vaccines, wound healing, treatment of ischemic tissue and ablation of tumors. One shortcoming that is often highlighted is the need to administer electric pulses at amplitudes that induce discomfort. To overcome this issue, we have explored means to reduce the applied voltage and/or number of pulses needed to accomplish either delivery or ablation. One approach has been the addition of moderate heat which can increase the membrane fluidity of exposed cells and potentially make them more susceptible to the effects of the applied field. The concept is being tested across multiple cell types and tissues and has shown a consistent advantage with respect to lowering the electrical parameters. To test and evaluate this concept in the various electrically-based modalities, we developed *in vitro* methods to test and evaluate various protocols in both 2-D and 3-D cultures. Results indicate that the addition of moderate heat and raising the temperature to 43° C enables a reduction in applied voltage as well as a reduction in the number of applied pulses without effecting the therapeutic potential. This concept has also been translated and shown to also be effective in reducing the applied voltage and number of pulses when tested in *in vivo* models.