IBC Meeting Minutes
12.8.25

Present: Betty Jacques (Chair), Alison Klein, Jeramie Ellis, Bryce Abbey, Austin Nuxoll, Robin Harding, Chris Waples (ex officio)

Absent: Brad Krohn, Drew Prososki

Jacques (Chair) called meeting to order at 3:36 PM.

Minutes from Previous Meeting
Abbey (Nuxoll) moved to approve minutes from the October 13, 2025 meeting. Motion passed: 5-0.

Discussion of Protocols Under Review

REVISIT - Haiwei Lu – UNK-00001543 – Agrobacterium-mediated plant transformation

1. Should be P-BSL 1 (they are doing PPE as though they are BLS2 though)

Discussion

- Largely meets requirements for BSL2, but lacks automatically closing, locking door.
- Options may exist for partitioning the space to satisfy locked door.

Abbey (Ellis) moved to change required BSL classification from BSL2 to BLS1 with PPE. Motion passed: 5-0.

Yipeng Sui – UNK-00001551: Evaluation of the Dyslipidemic Impacts of a Universal Plasticizer Alkylsufonic Phenyl Ester

1. The use of C57BL/6 mice will allow us to test our hypothesis regarding the role of PXR in mediating ASE's dyslipidemic effects in vivo. We have chosen mice because they are a standard, well-recognized system for investigating cardiovascular diseases in vivo, and because by using mice we will have the opportunity to compare our results with the data presented in the large volume of pertinent, documented studies. Groups of 8-week-old male and female C57BL/6 wildtype mice will be orally gavaged (orally fed) with corn oil containing ASE at a dose of 10 mg/kg BW per day with or without PXR specific antagonist Resveratrol (45 mg/kg/day) for 7 days on a semisynthetic low-fat AIN76a diet containing 0.02% cholesterol.

   All mice will be euthanatized by ketamine/xylazine or carbon dioxide and then exsanguinated by left ventricular puncture followed by perfusion with heparinized saline and then removal of the tissues. Ketamine and xylazine are stored in the Chemistry Department Stockroom in a lock box and are checked out when needed to anesthetize mice prior to surgery. The amount of ketamine and xylazine used is documented to ensure proper use and following surgery, ketamine and xylazine are returned to the Stockroom for safe storage. The PI is the only person who can check out or return ketamine and xylazine for this project. The other reagents and chemicals used in this work are stored in

the proper location in the lab. All research personnel are trained and required by the PI to wear disposable lab gloves and lab coats when handling biohazards and chemicals. They are also trained to dispose of biohazard materials (e.g. items containing mouse blood/serum and tissue) in biohazard trash bags that are autoclaved before disposing.

Discussion

- Seek confirmation from investigator that ACE will not be heated, as it can become explosive at high temperatures.
- Resveratrol is an eye irritant in dust form, so eye protection should be worn.
- Biohazard bags should not be autoclaved.

Abbey (Nuxoll) moved to approve protocol, pending clarification of the above-listed concerns and resolution confirmation by IBC Chair. Motion passed: 5-0.

Catherine Johnson: UNK-00001536: Cell Culture of BSL-2 Human Cell Lines:

1. [Aim 1: Human Osteoblast Cell Line] The effects of enzalutamide, a small molecule inhibitor of androgen receptor (AR) will be tested on human hFOB1.19 osteoblasts cultured in vitro to analyze the osteoblast-specific effects of AR-targeted therapy. The effects of enzalutamide treatment on osteoblasts will be measured using viability assays, differentiation assays, RNA sequencing to measure transcriptomic changes with a focus on differential expression of secretome genes, and enzyme-linked immunosorbent assays (ELISAs).

   [Aim 2: Human Prostate Cancer Cell Lines] To determine the impact of therapy-treated osteoblast-derived factors on prostate cancer progression, human cell line VCaP will be cultured in conditioned medium from vehicle- or enzalutamide-treated osteoblasts. The effects of osteoblast-derived factors on tumor cells will be measured using migration/invasion, proliferation, and therapy dose-response assays.

Nuxoll (Abbey) moved to approve protocol. Motion passed: 5-0.

Austin Nuxoll: Amended UNK-00001474: Antimicrobial activity of Benzyl Isothiocyanate:

1. [Project 1: Determine Antimicrobial activity of benzyl isothiocyanate] To evaluate the in vitro antibacterial activity of BITC against various oral bacterial strains, (S. mutans, S. oralis, S. agalactiae, S. sobrinus, P. gingivalis, and F. nucleatum) will be screened for minimum inhibitory concentrations and minimum bactericidal concentrations. Many of these bacteria can form biofilms and the BITC compound will be tested against these bacteria when growing in this environment as they are notoriously difficult to treat while in a biofilm. [Project 2: Determine the toxicity of BITC to eukaryotic cells] Eukaryotic cell lines (RAW264.7 macrophages and L-929 fibroblasts) will be incubated with increasing concentrations of BITC followed by a trypan blue assay to determine cell viability as well as an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. To determine if BITC is mutagenic, an AMES test will be performed to determine if there is an increase in prototrophic mutants following exposure to BITC.

a. **The only change in this protocol is to add in a new cell line to test BITC for cellular toxicity. This is in addition to the RAW264.7 cell line mentioned in the Research Description. We will use CCL-1 NCTC Clone 929 Areolar Fibroblast Mouse available through ATCC. These are a BSL-1 cell line.**

Abbey (Ellis) moved to approve protocol. Motion passed: 4-0-1 (Nuxoll abstaining)

Austin Nuxoll: Amended UNK-00001475: Efficacy or Plasma Activated Antibiotics against Antibiotic Tolerant Bacteria

1. [Project 1: Determine the mechanism(s) of plasma-activated antibiotics.] Previous studies have identified plasma-activated water as an effective treatment against a number of bacterial species. Additionally, studies have shown plasma-activated water leads to biofilm dispersal. In this project, we will characterize the oxidizing species present in plasma-activated antibiotics as well determine which species are responsible for antimicrobial activity. Characterization of plasma-activated antibiotics will occur through liquid chromatography with tandem mass spectrometry (LC-MS-MS) and subsequent nuclear magnetic resonance (NMR) spectroscopy analysis. Scavengers of reactive oxygen and nitrogen species (RONS) will be added to biofilms prior to plasma-activated antibiotic treatment with subsequent RONS measurement through CM-H2DCFDA and methylamino-2,7-difluoroflurescein dyes. [Project 2: Determine efficacy of plasma-activated antibiotics in vivo.] Antibiotic tolerant cells and biofilms are notoriously difficult to treat through conventional antimicrobial therapy. Recent work in our lab demonstrated the inability of antibiotics to kill biofilms due to persister cells. A Drosophila melanogaster biofilm infection model, a Zophobas morio infection model, and a Galleria mellonella infection model will be utilized to determine whether plasma-activated antibiotics are effective in targeting S. aureus within a host. Following infection of both invertebrates, plasma activated antibiotics will be injected and survival will be monitored over 2 weeks. We will use CCL-1 NCTC Clone 929 Areolar Fibroblast Mouse cells to test whether the plasma activated antibiotics are toxic to eukaryotic cells using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay.
    a. **I will be adding a different cell line to test the plasma activated antibiotics for eukaryotic cell toxicity. It is a BSL-1 cell line and we will be using CCL-1 NCTC Clone 929 Areolar Fibroblast Mouse cells. We have been using Z. morio for the infections but have run into consistency issues. Since more studies use Galleria mellonella for infection with bacteria, we plan to use this species which is available through Carolina Biological. We are also adding a new student to the protocol.**
    b. **Eye protection will be worn when inoculating worms as well as when bacterial burden is taken at the end of the experiment. This procedure involves homogenizing the worms and this has risk of splashing. Needles will be discarded in a sharps container, and they will not be recapped.**

Abbey (Ellis) moved to approve both above-listed protocols (UNK-00001474 & Amended UNK-00001475). Motion passed: 4-0-1 (Nuxoll abstaining)

Other Business

Robin Harding expressed interest in continuing to serve on the IBC, after her retirement from full-time employment at UNK.

UNMC Lab Coordinator (Stan Iliff) has expressed some interest in serving on the IBC. Thoughts?

- Members broadly expressed support for his membership, with emphasis on his expertise with lab management.

New supervisory role being added to Facilities. Has safety/EHS experience.

Abbey (Nuxoll) moved to adjourn meeting at 4:07 PM.