ALTERNATIVES
RESEARCH & DEVELOPMENT
FOUNDATION

# 2019 ARDF GRANT RECIPIENTS

*Established in 1993, the ARDF has been a mainstay of support for developing alternatives to animal-based methods in science. Through grant programs, achievement awards, and sponsorship of scientific conferences, ARDF advances high quality scientific research that aims to replace and reduce the use of animals.*

## 1 ABHINAV BHUSHAN, PH.D.

*Department of Biomedical Engineering, Illinois Institute of Technology, Chicago, IL*

**Microbe induced perturbation of drug metabolism through an intestine organ-on-chip**

Nowhere is the use of animals for research more wasteful than in the study of the microbiome and the gut. Not only is the physiology of the animal gut very different from the human gut; animal microbiome is dissimilar from that of humans. Therefore, despite the importance of intestine-bacteria in drug metabolism [bacteria can enhance/diminish the efficacy and toxicity of drugs], animal studies are impeding the translation of results to humans. This is primarily because the gut microenvironment is challenging to capture in vitro. Considering that the potential impact of millions of bacteria on the hundreds of drugs, the non-availability of an animal-free in vitro platform could result in the unnecessary sacrifice of millions of animals. To address this challenge, the focus of this proposal is to develop novel animal-free cell-based intestinal models to study the influence of microbiota on drug metabolism. The biggest impact of this work will be the resulting in vitro platform that will reduce the usage of animals for all researchers, saving millions of lives. Understanding how bacteria can affect drug metabolism and disposition can open-up novel bacteria and/or molecular pharmaceutical-directed approaches to optimize enzymatic and transporter activity of therapeutic agents.

## 2 COLIN E. BISHOP, PH.D.

*Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston-Salem, NC*

**Multi cellular, 3D Human Liver Organoids as a Model for Nonalcoholic Steatohepatitis**

Nonalcoholic fatty liver disease (NAFLD) is the accumulation of triglycerides and free fatty acids in the liver. Nonalcoholic steatohepatitis (NASH) is the progressive form of NAFLD and is characterized by liver steatosis, inflammation, hepatocellular injury and fibrosis. NAFLD is rapidly becoming the most common chronic liver condition in Western populations (34% in the US) and the prevalence of NASH (currently 12% in the US) is expected to increase by 63% between 2015 and 2030. To date, there are currently no approved agents for treating NAFLD/NASH, thus efforts to identify new therapeutic drugs is extremely urgent. Currently, potential drugs are tested using rodent and primate models of NAFLD/NASH. However, such screening systems are slow, expensive, not fully predictive of the human state and use an alarming number of animals. The multicellular, 3D human liver organoid system we propose to develop here represents an alternative to such testing. As all the cells are primary human liver cells, they should have a much higher predictive value than animal models. Finally, such a high throughput system will allow the screening of hundreds of different classes of potential drugs acting on a variety of targets without the use of animals.

## 3 RAYMOND CHA, PHARM.D. & BRIAN TSUJI, PHARM.D.

*Laboratory of Antimicrobial Pharmacodynamics, The University at Buffalo, Buffalo, NY*

**Validation of a Mini-In Vitro Pharmacodynamic Model with Human Serum Constituents and Albumin**

The increasing prevalence of multiple-drug resistant organisms in the face of a diminished antimicrobial pipeline devoid of agents with novel mechanisms of action, necessitates an enhancement of current pharmacologic evaluations beyond bacterial kill. The potential loss of a last-line antimicrobial like carbapenems emphasizes the urgency for inexpensive in vitro systems to maximize evaluations. We propose a novel in vitro model that incorporates human serum constituents and albumin that may be poised to reduce the need for murine infection models. This in vitro pharmacodynamic model simulates human antimicrobial concentrations and allows optimized sampling for antimicrobial resistance assessment. The proportions of resistant bacterial populations that express genes that confer carbapenem resistance will be determined over time as a function of dose range and alternate infusion strategies. We will develop quantitative system pharmacology models that will enhance the framework for antimicrobial resistance metrics in antimicrobial dosage and schedule design. This in vitro platform can be extended to include other immune constituents and drug-related biologics.

## 4 SARA FORTUNA, PH.D. & MIGUEL SOLER, PH.D.

*Department of Chemical and Pharmaceutical Sciences, University of Trieste, Trieste, Italy*

**In silico design of customised high-affinity antibody fragments**

We aim to show that our in silico method of binder design can compete and eliminate the demand of in vivo procedures for antibody discovery and maturation. The huge increase of medical applications involving immunoreagents urges both industry and academia to employ effective methods for antibody discovery. Methods that often heavily rely on the use of animal immunogenic response. The present technology is compatible with the ex-novo computational optimization of single-domain antibodies (VHHs). In this framework our team has developed a new enhanced computational protocol to improve the binding affinity of a panned VHH by one order of magnitude by optimizing one single CDR. Our recently validated affinity optimization algorithm will be shown to be superior to the current state of the art techniques through the ex-novo optimisation of VHHs by: (i) defining an optimum protocol by first targeting well known system, (ii) generating binders for a biomarker of medical interest associated with cancer recurrence prognosis, (iii) using the generated binders for the development of new nanodevices for biomarker detection. Our multidisciplinary team can thus lay ethically sound design principles for the development of novel binders. This work marks the first step towards the in silico evolution of full antibodies.

## 5 LI JIN, PH.D. & XUDONG JOSHUA LI, PH.D

*Orthopaedic Surgery, University of Virginia, Charlottesville, VA*

**A Bioprinting Human Disc Organoid-on-a-Chip Platform to Tackle Degenerative Disc Disease**

Low back pain / intervertebral disc degeneration is the most common problem with a prevalence of 80% and an annual cost of $100 billion in the U.S. Unfortunately, no disease modifying treatment is yet available. The lack of a high throughput platform hampers the advancement of drug discovery to tackle degenerative disc diseases. Thus, spine related research is booming.

The mainstream models for disc degeneration use animals, whereas human discs differ significantly from animal discs. Our goal is to create a scalable human disc-organoid-on-a-chip platform by integrating 3D bioprinting and microfluidic technologies.

1. Bioprinting human disc organoid will minimize animal usage by using human cells and will be an invaluable biomimetic tool for high throughput drug discovery and toxicity assessment.

2. The microfluidic device will only need 1/16 of culture media of conventional culture system, thus remarkably reduce animal serum usage.

The “disc-organoid-on-a-chip” device can be customized and manufactured in mass production and utilized by all of research communities, shifting the paradigm of conventional animal research to a human organoid model and accelerating drug discovery for this devastating condition. In addition, this platform is readily transferable to other fields, such as cancer and other chronic diseases.

6 MELISSA JONES, PH.D. & MARIOLA EDELMANN, PH.D.
*Microbiology and Cell Science Department, University of Florida, Gainesville, FL*

**Evaluating and modifying exosome production and content to improve human norovirus cultivation in B cells.**

Our understanding of human noroviruses (HuNoVs) has been hampered by the lack of robust in vitro cultivation systems. Several cell types support HuNoV replication in vitro but only produce modest viral titers which are insufficient for use in drug development and use in foundational studies investigating HuNoV pathogenesis. This has led to a reliance on mouse and other animal models to gain insight into this human pathogen. The reasons for the low level HuNoV replication in vitro are unknown. However, the widespread role of exosomes in viral infection and the recent discovery of exosome use as a means of norovirus escape from infected cells may provide some insight. These vesicles are a well-known and potent means of intercellular communication. We hypothesize that noroviruses enhance exosome production during infection to facilitate viral egress but have a secondary effect of carrying signaling molecules to naïve cells, rendering them non-permissive to infection. This scenario would ultimately result in the low viral output characteristic of HuNoV cultivation. Our project will evaluate exosome production in B cells infected with HuNoVs, characterize the content of the exosomes produced, and determine if exosomes contribute to reduced HuNoV replication in vitro.

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