ALTERNATIVES
RESEARCH & DEVELOPMENT
FOUNDATION

2023 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 VINCENZO CIRULLI, MD, PHD AND LAURA CRISA, MD, PHD

University of Washington, Seattle, WA

Modeling diabetes in an organoid on chip platform

Stem cells hold a great potential to cure Type 1 Diabetes (T1D), an autoimmune disease that causes the loss of insulin-producing ß-cells1. Recent advances led to the development of protocols that produce unlimited numbers of ß-cells from human pluripotent stem cells (hPSC). Yet, despite promising results from recent pilot clinical trials2, significant gap in knowledge remains on the molecular mechanisms driving the functional maturation of hPSCs into pancreatic ß-cells3. Thus, the secretory function of these hPSC-derived islet cells requires further maturation before acquiring the competency to secrete insulin in response to glucose. Presently, the endocrine function of hPSC-derived islet cells is tested in vitro, and in vivo after transplantation in animal models. Both of these methods have their advantages and limitations. While the in vitro testing can assess their competency to secrete insulin in response to glucose, these islet-like cell clusters lack critical tissue components found in pancreatic islets in vivo such as the vasculature, known to play a critical role in regulating insulin secretion. Conversely, testing their function in vivo after transplantation, although adding the contribution of the host's vasculature, requires long times for cell engraftment (10 days to three weeks) and makes use of large numbers of animal models. In this project we will test the ability of islet organoids-on-chip to recapitulate in vivo-like tissue microenvironments that integrate hPSC-derived islet tissue, endothelial cells and specialized extracellular matrices (ECMs) that we have identified to play a critical role in islet cell differentiation and function. Based on our pilot experiments, we anticipate that our pancreatic islet organoid-on-chip will not only provide a transformational new platform to model diabetes in vitro for testing the function of hPSC-derived islet tissue, but could also replace animal testing all together for the identification of new therapeutics.

2 QUINN EASTER, PHD AND KEVIN BYRD, DDS, PHD

ADA Science & Research Institute, LLC, Gaithersburg, MD

Precision-cut gingival slices as an animal-free model of oral inflammation

Periodontitis is the most common oral inflammatory condition, caused by chronic dysbiosis at the epithelial barrier and affecting ~50% of adults. Despite this significant burden, attempts to bring precision treatments have had minimal success to-date due to the inherent complexity of periodontitis. It remains unclear how these cells interact in health and disease. Further, we lack good models that conserve human tissue anatomy to determine how these cells function in health and contribute to disease pathogenesis. We need new methods to dramatically reduce or replace animal models of disease. Fluorescent in-situ hybridization (FISH) methods such as Xenium have emerged as powerful antibody-free toolkits to spatiotemporally investigate cell-specific gene expression patterns. Implementing such RNA detection techniques would dramatically reduce our reliance on animal-derived antibodies for immunofluorescence and other applications. To better understand periodontitis in an animal-free manner, this proposal will utilize two novel methods: 1) growing precision-cut gingival slices (PCGS) to evaluate tissue anatomy; 2) challenging PCGS with bacterial periopathogen lipopolysaccharides (LPS) to evaluate tissue cytokine C-X-C and C-C motif and interleukin signaling, analyzed by Xenium. Aim 1 will evaluate cell identity maintenance after culturing the PCGS. Aim 2 will utilize PCGS to model disease progression in periodontitis. Bioinformatics analysis will define cellular neighborhoods in gingiva, within insight into neighborhood change during disease pathogenesis. We hypothesize that 1) epithelial cell chemokine and interleukin signaling patterns will reveal the earliest events of dysbiotic-driven inflammation and 2) an animal product-free model of induced inflammation will be generated. Once successful, this system can be 1) utilized to replace some animal models of periodontitis and 2) expanded to other common and even rare, oral inflammatory diseases.

3 KSHITIZ, PHD AND JUNAID AFZAL, MBBS, MS

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Highly matured human cardiac models to measure cardiotoxicity

Cardiovascular toxicity is the leading cause of drug attrition in preclinical stages of drug development. Current methods to screen cardiotoxicity involve small rodent models, and in later stages, larger animal models, including pigs, dogs, and monkeys. Human adult cardiomyocytes (hACM) fundamentally differ from rodent myocytes at molecular, structural, electrophysiological, and metabolic levels, rendering them as poor proxies for hACMs. FDA, and National Center for Toxicological Research (NCTR) have recognized these widespread scientific considerations, stating an urgent need to develop human relevant cardiac platforms. Although human induced pluripotent stem cells (hiPSC) derived cardiomyocytes (hiPSC-CMs) were heralded with great promise as preclinical drug toxicity models, their potential is yet to be realized because differentiated hiPSC-CMs are immature, are at early fetal stage, and do not reflect the characteristics of hACM. Process of limited maturation is very long (90 to 180 days), rendering them unviable, highlighting the importance of accelerated maturation of hiPSC-CMs measured against recognized hACM hallmarks.

We have developed a platform to rapidly accelerate hiPSC-CMs maturation by maintaining them on a substrate mimicking ligand chemistry, rigidity, and nanotopographic ultrastructure, Cardiac Mimetic Matrix (CMM). CMM results in rapid and robust maturation of hiPSC-CMs within 30 days with similar or superior structural, mechanical, and electrophysiological characteristics than much prolonged cultured (90 to 180 days), and vastly superior metabolic maturation and redox handling capability than current models.

In this targeted proposal, we will test the efficacy of CMM matured hiPSC-CMs on a panel of FDA recommended drugs with known electrophysiological effects on hACMs or large animal models, and test their interaction with alcohol induced toxicity, with the aim to develop CMM as an effective, and practical alternative to animal models.

4 MORITZ PFEIFFENBERGER, PHD

Charité Universitätsmedizin Berlin, Berlin, Germany

First steps towards a fracture hematoma on-a-chip model – Mimicking the initial phase of fracture healing in vitro

Musculoskeletal disorders are among the leading cause of years lived with disability for millions of people worldwide due to the increase in life expectancy and an aging population. Direct and indirect costs of illness constitute a considerable socioeconomic burden. Fracture healing disorders are disabilities associated with pain and therapeutic interventions occurring in approximately 10% of fractures. Despite progress in the treatment of fracture healing disorders, a strong unmet medical need remains, as adequate patient care is only possible if new therapeutic strategies pass the preclinical and clinical phases. In the last years, we have witnessed the failure of potential new therapies in clinical trials, although their development was based on promising animal data. Particularly in the preclinical phase, the use of animal models is still common, as no appropriate in vitro model exists which can mimic the (patho)physiological relevant environment of fracture healing, thereby including all cells and signaling molecules.

Within a previous project, a human-based in vitro 3D model of the initial phase of fracture healing was established, clearly demonstrating the suitability of the model in distinctly mimicking key characteristics of the initial phase of fracture healing. In brief, we first characterized a fracture hematoma model consisting of a coagulate of human peripheral blood and mesenchymal stromal cells, known to be a crucial player in terms of an appropriate healing process. In parallel, we characterized a bone model consisting of condensed MSCs and could show striking similarities to native bone. Finally, we combined both models and could show distinct overlaps to the initial phase of fracture healing. In the follow-on project proposed here, we aim to develop the first steps towards scaling up the model and creating a high-throughput system using state-of-the-art organ-on-a-chip technology, thereby significantly improving the accessibility for other users.

5 MAREN SCHENKE, PHD

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Advanced neurotoxicity testing with an in vitro model of sex differences

In vitro models are becoming increasingly complex, predictive, and more representative of human physiology. However, differences between males and females, which are present in many aspects of human physiology, are hardly ever addressed and modelled in vitro. The development of the brain is sex-specific, which may manifest in different sensitivities of males and females to neurotoxicants. Furthermore, the incidence of many neurological disorders is also sex-dependent (e.g. the autism prevalence ratio female:male is 1:4). This might be due to increasing exposure to not sufficiently studied chemicals in our environment, such as phthalates, which are used as plasticizers in articles of everyday use.

The roots of neurological sex differences can be found in the foetal development, where androgens induce the masculinization of the male human brain, while the female brain mainly develops in the absence of sex hormones. In rodents, however, estrogens are responsible for the masculinization of the male brain, decreasing the suitability of rodents as a model. While the pathways involved in the sexual differentiation of the brain have hardly been studied in humans, we are exposed to endocrine disrupting chemicals as well as chemicals that induce developmental neurotoxicity. Testing in animals is not only prohibitively expensive and requires an enormous number of animals, but also not representative of human brain development.

While these two classes of adverse effects are beginning to be covered by in vitro assays, their intersection has been hardly investigated. We propose to study the implementation of sex hormones in a model of the human brain, to identify suitable biomarkers of sexual differentiation in vitro and how it is disturbed by endocrine disruptors during development. We will investigate the effects of the exposure to an endocrine disruptor, the phthalate DEHP on the masculinization processes, aiming to lay the groundwork to improve current in vitro assays.

6 VENKATARAMANA SIDHAYE, MD

Johns Hopkins University, Baltimore, MD

Developing a cigarette smoke exposure model using human precision cut lung slices to study COPD and potential drug therapeutics

Chronic respiratory diseases impact over 500 million people globally. Chronic obstructive pulmonary disease (COPD) accounts for up to 50% of prevalent cases of chronic respiratory disease. Despite its large impact on global health there are no pharmaceutical interventions that successfully stop or reverse disease progression. There is also a high failure rate of clinical trials targeting respiratory diseases which may be driven by an overreliance on animal models for pre-clinical data. Animal models of COPD, and other chronic lung diseases, may be able to recapitulate certain phenotypic characteristics of human lung disease but are limited by differences in lung size, structure, and cellular composition. Additionally, standard methods to induce COPD (e.g., cigarette smoke exposure, elastase instillation) in animal models require weeks to months of exposure, contributing to a considerable amount of stress and distress in the animals used. To improve the human relevance of pre-clinical data, and reduce reliance on animal models, we propose the use of living three-dimensional precision-cut lung slices (PCLS), as a platform to model COPD and screen potential therapeutic compounds. PCLS allows for the investigation of lung disease in structurally relevant regions of the lungs, where all native cell types, extracellular matrix, and architecture are present. Recently, we have adapted our validated in vitro cigarette smoke exposure system to accommodate PCLS, allowing us to recapitulate COPD phenotypes in PCLS from healthy donor lungs. Through the proposed research we will 1) optimize a cigarette smoke exposure regimen to induce COPD-like alterations in PCLS and 2) develop standard procedures to test the efficacy and potential toxicities of drug candidates to treat COPD. Successful completion of this study will validate a human-relevant platform that will expand knowledge of respiratory disease and advance drug testing while reducing reliance on non-human animal models.

7 MATHIEU VINKEN, PHD

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A human-relevant and AOP-driven in vitro approach for studying the cholestatic potential of micro- and nanoplastics

Plastics are versatile materials with a myriad of applications in daily life and degrade in the environment to smaller fragments called micro- and nanoplastics (MNPs). Although still controversial, MNPs have been associated with a variety of adverse health effects. Recent animal studies showed alteration of bile acid homeostasis in the liver induced by MNPs, which suggests cholestatic potential. However, animal testing in the MNP field raises serious ethical questions and has been frequently criticized because of the lack of human relevance. This defines the scope of the present project, in which it will be investigated if MNPs not only induce bile accumulation, but also exacerbate pre-existing cholestasis in human liver. For this purpose, a human-centric in vitro setting will be set up consisting of liver-based cell culture systems linked with a series of assays, each that monitor one or more mechanistic key events in the adverse outcome pathway network of cholestatic liver injury at the transcriptional, translational or functional level. Cell culture systems allowing prediction of cholestatic adversity at the personalized and general population level will be used, and will be exposed to MNPs in in vivo-like and human-relevant conditions. Overall, this project will shed more light onto the liver toxic potential of MNPs and will demonstrate the power of animal-free and human-based in vitro experimentation for hazard identification purposes.

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