HARVARD CATALYST

THE HARVARD CLINICAL AND TRANSLATIONAL SCIENCE CENTER

Big Ideas, Small Features: Utilizing Advanced Microscopic and Nanoscale Technologies to Further Human Healthcare

Funded Projects

This initiative of the Harvard Catalyst Reactor Program provided funding of up to $50,000 in funding and access to the Harvard Center for Biological Imaging (HCBI), which features advanced Zeiss microscopes, or the Center for Nanoscale Systems (CNS), that offers electron microscopy, nanoscale fabrication, and nanoscale analysis capabilities, or access to both. Funding will support the innovative application of light and electron microscopy, nanoscale fabrication, and nanoscale analysis technologies for big ideas that will advance clinical healthcare.

The goal of this opportunity was to support innovative research projects that could provide new insights into: (1) the application of new technologies to inform clinical decisions; (2) disease detection, causation, progression, or treatment; or (3) the development of new therapeutics, diagnostics, or clinically informative biomarkers.

This funding opportunity was only open to investigators who attended a training/educational event or met with Harvard Catalyst personnel.

Eight pilot grants were awarded in amounts of up to $50,000 for each one-year project.

Funding decisions were announced in August 2016.

Injectable Hydrogel Delivers miRNA-therapeutics to 3D-Bioprinted Calcific Aortic Valve Disease Model

Principal Investigator: Elena Aikawa, MD, PhD, Brigham and Women's Hospital

Co-Investigator: Casper van der Ven, MSc, Brigham and Women's Hospital

Calcific aortic valve disease (CAVD) claims 17,000 lives in the US alone, annually. No drug-based therapy is available. The only effective treatment is invasive and costly aortic valve replacement for late-stage disease patients. This study will use a novel 3D-bioprinted model of CAVD and an innovative drug-delivery platform to elucidate the underlying pathobiological mechanisms of CAVD and identify potential therapeutic targets.

Advanced imaging and nanoscale technologies at the HCBI and CNS will greatly enhance our insight into mechanisms of CAVD. To illuminate the pathobiological mechanisms of CAVD, a 3D-bioprinted CAVD model will be used to track differentially expressed microRNAs recently identified in our screening of CAVD tissues. The bioprinted CAVD model will allow us to assess the temporal association of these microRNAs with cellular changes observed in CAVD using fluorescent *in situ* hybridization and fluorescent phenotypic markers. We will then test whether these microRNAs can be targeted to therapeutically control CAVD cell phenotypes. Confocal microscopy will track microRNA delivery from a hydrogel drug delivery system into cells incorporated into CAVD model. Visualizing microcalcifications in the CAVD model using a near-infrared fluorescent calcium tracer will assess the functional response of calcified cells to microRNA-therapeutics.

This research will validate an innovative injectable hydrogel delivery platform in a unique 3D-bioprinted model of CAVD and identify functional combinations of miRNA therapeutics with a main goal to improve the standard of care for patients suffering from CAVD, a disease with no available drug strategies.

Advancing Coil Design in Micromagnetic Brain Stimulation

Principal Investigator: Giorgio Bonmassar, PhD, Massachusetts General Hospital

Micromagnetic stimulation (µMS) has several advantages over electrical stimulation. First, µMS does not require charge-balanced stimulation waveforms as in electrical stimulation. In µMS, neither sinks nor sources are present when a current is induced by the time-varying magnetic field, thus µMS does not suffer from charge buildup as can occur with electrical stimulation. Second, magnetic stimulation via µMS is capable of activating neurons with specific axonal orientations. Moreover, as the probes can be completely insulated from the brain tissue, we expect to significantly reduce the problem of excessive power deposition into the tissue during magnetic resonance imaging (MRI). µMS technology was first developed in our laboratory and is entirely based on commercial components off the shelf, which are readily available to researchers. However, commercially available inductors are designed to maximize efficiency (Q-factor), which consists in trapping the generated magnetic field to minimize its losses. Furthermore, such coils do not allow for multiple coil design in small and complex 3D geometries as it is often needed in neuroscience applications. In this application we propose to acquire new thin-film technology at the Center for Nanoscale Systems (CNS) Harvard University to acquire the know-how and expertise to design, fabricate and test nanoscale coil structures for next generation of µMS devices. Such devices could become potentially the pacemakers and brain stimulators of the future with their contactless ability to deliver the neuronal stimulation

needed for therapeutic efficacy for patients in need of implantable cardioverter-defibrillators or pace-makers, or in patients with Parkinson's disease, epilepsy, and major depression.

Optimization of Drug Delivery by Tumor-Targeting Layer-by Layer Nanoparticles Using Advanced Microscopic Technologies

Principal Investigator: David Frank, MD, PhD, Dana-Farber Cancer Institute

Continuing advances are being made in the development of novel therapies for cancer. However, the ability to target anti-cancer drugs directly to tumor tissue remains a significant unmet need. In addition, it is becoming increasingly clear that delivery of combinations of targeted agents in a defined temporal sequence, such as inhibitors of BRAF and MEK for the treatment of melanoma, is extremely important for the efficacy of these therapies. Tumor cells have unique physiochemical properties, including altered composition of their cell membranes, which provides an opportunity that can be exploited for drug delivery. We have been working to develop a unique drug delivery system, known as layer-by-layer (LbL) nanoparticles, that can both deliver drugs with high efficiency to cells with specific membrane characteristics, as well as deliver more than one drug with a defined temporal sequence. However, it is extremely difficult to characterize the delivery characteristics of the multiple layers of an LbL nanoparticle to tumors. Access to the imaging capabilities of the Harvard Center for Biological Imaging provides a unique opportunity to address these questions. We propose to package distinct fluorophores in the layers of LbL nanoparticles of diverse design and use advanced microscopic technologies to quantitate the delivery to tumor model systems. These experiments will have significant translational implications, and will provide insight into basic properties of cancer cell membranes. Based on the findings of these studies, we would plan to perform experiments in orthotopic model systems in animals, as a prelude to therapeutic clinical trials in patients.

A Microscopy-Based Platform For Rapid, At-will Antimicrobial Resistance Testing

Principal Investigator: James Kirby, MD, Beth Israel Deaconess Medical Center

Co-Investigator: Kenneth Smith, PhD, Beth Israel Deaconess Medical Center

Antibiotic resistance is compromising our ability to treat bacterial infections. Clinical microbiology laboratories guide appropriate treatment through antimicrobial susceptibility testing (AST) of patient isolates. However, increasingly, pathogens are developing resistance to a broad range of antimicrobials, requiring AST of less commonly used or recently introduced agents for which no commercially available or FDA-cleared testing methods exist. Agar or broth dilution are gold standard methods for AST that can be used to test any antimicrobial; however, labor and technical complexity precludes their use in hospital-based clinical laboratories. Therefore, bacterial isolates often must be sent to a reference laboratory with a 4-6 day delay in results. Further, even standard methods require overnight incubation prior to readout. Therefore, there exists a significant AST testing gap in which current methodologies cannot adequately address the need for rapid results in the face of unpredictable susceptibility profiles. Our laboratory has recently verified inkjet, digital dispensing technology as a novel platform to facilely perform reference AST for any antimicrobial at will. In this proposal, we aim to harness technical assets and expertise at HCBI/IDAC to leapfrog current technology through: (1) development of a method for microscopic imaging of bacterial replication in solid-phase 384-well microplate AST format, thereby determining susceptibility for any drug in <4 hours and (2) verification of the clinical performance of the new assay using well-characterized clinical

isolates. We anticipate establishing a prototype method that will address the AST testing gap and thereby help our health system more effectively address the antimicrobial resistance threat.

Laser-activated Plasmonic Intracellular Delivery: Using Micropyramids to Deliver CRISPR-Cas9 to Hematopoietic Stem Cells (HSCs)

Principal Investigator: Eric Mazur, PhD, Harvard School of Engineering and Applied Sciences

We propose 1) to investigate how our laser-activated intracellular delivery technique affects living cells and 2) to deliver genome-editing biomolecules to living cells for human health-care applications. The safe and efficient delivery of macromolecules to living cells is crucial for advancing health care. No currently available intracellular delivery method concurrently offers the characteristics necessary for successful therapy: high efficiency, viability, and throughput; delivery of diverse cargo into diverse cell types; spatial selectivity; and scalability. We pioneered the use of laser-activated micropyramids that absorb light and generate microbubbles to porate the cell membrane, allowing membrane-impermeable macromolecules to diffuse into the cell. Our competitive non-viral intracellular delivery technique simultaneously offers all desirable features: preliminary results show 90% delivery efficiency, 97% viability and 10,000 cells/s throughput with HeLa cells. Our team has the requisite expertise in nanofabrication, laser physics, and cell biology to propel our technology to positively impact biomedical and human heath-care research. The Catalyst grant will enable us to combine our current nanofabrication and cell culture research at CNS with advanced imaging at HCBI to investigate cellular response and to deliver genome-editing tools to living cells using our technique. With the support of the Catalyst team, we will develop our technique as a first step towards creating treatments for genetic diseases.

Metabolic Imaging of Mouse Embryos to Determine Safety of 1-Photon FLIM for Clinical Applications in *In Vitro* Fertilization

Principal Investigator: Daniel Needleman, PhD, Harvard University Faculty of Arts and Sciences

Mitochondrial dysfunction has long been associated with reduced reproductive potential. More than 200 publications link mitochondrial function with *in vitro* fertilization (IVF) success. 67% of all IVF cycles fail, making the process economically and emotionally costly to patients and the health system. Developing an effective and accurate embryo selection tool has long been a primary goal in clinical reproductive research, as it would have a dramatic impact on IVF success rates. Non-invasive assessment of mitochondrial health could provide the means to such a tool.

We have established that we can non-invasively assess mitochondrial function of oocytes by measuring NADH and FAD fluorescence using Fluorescence Lifetime Imaging Microscopy (FLIM). Experiments thus far have been performed on a 2-photon system in the Needleman Lab, and we have demonstrated the safety of 2-photon FLIM for use in oocytes and embryos.

The aim of this proposed research is to assess the safety and feasibility of a 1-photon FLIM system for generating FLIM measurements of NADH and FAD. To accelerate translation of our research to the clinical realm, we must better understand phototoxicity of 1-photon microscopy systems to determine whether they are clinically viable. We will achieve this aim by varying

photodosage for mouse oocytes and mouse embryos, and by measuring Reactive Oxygen Species levels, potential DNA damage, and live birth outcomes.

Massively Multiplexed *in situ* Imaging with Kinetic Barcoding

Principal Investigator: William Shih, PhD, Dana-Farber Cancer Institute

Co-Investigator: Douglas Richardson, PhD, Harvard University Faculty of Arts and Sciences

It would be enormously useful towards personalized medicine to be able to screen tumor biopsies by imaging them for thousands of biomolecular markers simultaneously, as this would enable rapid and cost-effective characterization of the tumor. Unfortunately, current methods in fluorescence microscopy are limited to only about four colors at one time. We propose an alternative strategy that offers both compact labels and rapid decoding of potentially thousands of distinct "colors": molecular barcodes that encode their "colors" (i.e. unique identities) based on prescribed blinking patterns, kind of like a molecular Morse code. We will implement these using DNA polymerases that move along designed templates of varying lengths. For this pilot grant, we propose to image cells and tissues using state-of-the-art instruments at the Harvard Center for Biological Imaging, such as FAST confocal and light-sheet fluorescence microscopes.

Uncovering Molecular Features Associated with DNA Copy Amplifications

Principal Investigator: Johnathan Whetstine, PhD, Massachusetts General Hospital

Co-Investigator: Shannon Stott, PhD, Massachusetts General Hospital

Genome instability and drug resistance are hallmark features of cancer. We have recently uncovered the first enzyme KDM4A capable of generating transient site-specific copy gains of regions linked to drug resistance and hard to treat cancer, which provides a novel tool to carefully interrogate the molecular features affiliated with copy gains. In this application, we are developing methods to characterize the genomic features associated with copy amplification. We will develop microscopy-based strategies to characterize factors that promote these events as well as the impact that copy gains have on gene expression. We are also developing microfluidic devices to isolate the copy gained DNA for sequencing analyses. Our proposed studies will shed light on basic regulatory mechanisms influencing genome organization and expression, while identifying novel biomarkers associated with amplification. Ultimately, we hope that the combination of the molecular insights gained from this pilot study combined with the microfluidic DNA sorting technology will enable the broad dissemination of these assays such that site-specific copy gains can be used to inform clinical designs and help improve patient outcomes.