2020 BIOSciTech SYMPOSIUM

REGENERATIVE MEDICINE: CRYOPRESERVATION OF CELLS TO ORGANS
Co-chairs: Mehmet Toner, Ph.D., Director, Center for Engineering in Medicine, Massachusetts General Hospital; Allison Hubel, Ph.D., Professor, Mechanical Engineering

10:30-10:35 Opening Remarks
10:35-11:05 EXTENDING THE LIMITS OF ORGAN PRESERVATION
Korkut Uygun, Ph.D., Associate Professor of Surgery (Bioengineering), Harvard Medical School and Massachusetts General Hospital
11:05-11:25 CRYOPRESERVATION AND THE SUPPLY CHAIN NEED FOR CELL THERAPY AND REGENERATIVE MEDICINE COMPANIES
Allison Hubel, Ph.D., Professor, Mechanical Engineering
11:25-11:45 MICROFLUIDIC ORGANOID CULTURES FOR PERSONALIZED TREATMENT OF CANCER
Alexander Revzin, Ph.D., Professor of Biomedical Engineering, Mayo Clinic
11:45-12:05 CELL, TISSUE, AND ORGAN CRYOPRESERVATION—COMMON APPROACHES AND UNIQUE CHALLENGES
Erik Finger, M.D., Ph.D., Department of Surgery, Division of Transplantation
12:05-12:30 Open Discussion
[12:30-12:55 BREAK]

AQUATIC SPECIES CRYOPRESERVATION
Co-chairs: Mary Hagedorn, Ph.D., Research Scientist, Smithsonian’s National Zoo & Conservation Biology Institute; John Bischof, Ph.D., Director, IEM

12:55-1:00 Opening Remarks
1:00-1:30 AQUATIC SPECIES CONSERVATION
Mary Hagedorn, Ph.D., Senior Research Biologist, Smithsonian Conservation Biology Institute/Hawaii Institute of Marine Biology
1:30-1:50 CRYOPRESERVATION AND LASER NANOWARMING OF AQUATIC SEED FOR CONSERVATION AND AQUACULTURE
Kanav Khosla, Ph.D., Mechanical Engineering
Kieran Smith, M.S., Research Scientist, Cryoocyte
1:50-2:10 CRYOPRESERVATION IN THE CONTEXT OF EMBRYOGENESIS AND ANIMAL EVOLUTION
Alex Primus, DVM, Ph.D., Assistant Professor, Department of Veterinary Population Medicine
2:10-2:30 DIRECTING SUBZERO TOLERANCE TO TEMPERATURE SENSITIVE ZEBRAFISH
Shannon Tessler, Ph.D., Instructor, Massachusetts General Hospital and Harvard Medical School
2:30-3:00 Open Discussion
EXTENDING THE LIMITS OF ORGAN PRESERVATION

Organ preservation is undergoing a revolution, with machine perfusion technologies rapidly expanding to the clinic in many vital organs, with the primary goal of enabling the transplantation of injured and currently unused organs to address the need for more donor organs. Perfusion serves also as an ideal entry point for more sophisticated preservation technologies into clinic. This talk will focus on machine perfusion as an enabling, platform technology for whole organ preservation and banking, including its utility as a controlled cryopreservation agent loading mechanism, value to provide treatment from cold ischemia after storage and prepare grafts for transplantation, and a key function in assessment of the organs for transplantation safely. The talk will also focus on the exciting new developments in high subzero organ preservation and scale up of these technologies to whole human organs.

Korkut Uygun, Ph.D.
Associate Professor of Surgery (Bioengineering), Harvard Medical School and Massachusetts General Hospital

Korkut Uygun, PhD, as built a major program for reengineering organs, specifically livers, aimed at ensuring donor organs are utilized for public good. PhD work focused on integrating process design and control, and identifying threat events for petrochemical process industries, specializing in model predictive control and dynamic optimization. In 2006, he joined the Center for Engineering in Medicine as a postdoc for training on liver surgery and transplantation. In 2008, he won the NIH Pathway to Independence Award that funded a project to recover unusable organs for transplantation, and predict graft survival success by developing metabolic models of the liver. Dr. Uygun’s lab (supported by NIH, NSF and Shriners Hospitals for Children) features a vertically integrated transplantation program with small and large animal transplantation and human liver pseudo-transplantation, as well as mass spectrometry-based metabolomics and systems biology expertise. In order to focus on translation of the machine perfusion technologies developed in his lab, he has co-founded a company, Organ Solutions LLC, which is funded by two NIH small business grants.
**REGENERATIVE MEDICINE: CRYOPRESERVATION OF CELLS TO ORGANS**

Co-chairs: Mehmet Toner, Ph.D., Director, Center for Engineering in Medicine, Massachusetts General Hospital; Allison Hubel, Ph.D., Professor, Mechanical Engineering

**CRYOPRESERVATION AND THE SUPPLY CHAIN NEED FOR CELL THERAPY AND REGENERATIVE MEDICINE COMPANIES**

The supply chain for cell therapy and regenerative medicine products is different from other medical therapies (medical devices, drugs, etc.). Cells may be collected in one place at a given time and used in another place at a later time. Effective methods of preservation will be critical for clinical and commercial use of cell therapies and regenerative medicine products. A process for efficient protocol optimization and elimination of dimethylsulfoxide will be described. Novel methods for preserving cells will use naturally-inspired approaches to improve preservation of cells used therapeutically. The integration of this approach into clinical and commercial applications will also be described.

Dr. Hubel is a Professor in Mechanical Engineering at the University of Minnesota and Director of the Biopreservation Core Resource (BioCoR, www.biocor.umn.edu), a national resource in biopreservation. Dr. Hubel has studied both basic science and translational issues behind preservation. Her work spans from the study of molecular mechanisms of damage during preservation to the development of technology to improve preservation outcomes. This work has resulted in the formation of two startup companies: MesoFlow and Blue Cube. As Co-PI of the U of MN REACH hub (MN-REACH), she was involved in the development of a skills development program that linked the local I-Corps program with the REACH hub. This program now serves innovators across the campus. Dr. Hubel has long been involved in faculty professional development was a founder the Big Ten Women’s Workshop. Dr. Hubel has published many scientific articles on preservation and she is the author of, “Preservation of Cells: a Practical Manual” published in 2018. She is a former deputy editor of *Biopreservation and Biobanking* and received the Outstanding Achievement in Biobanking Award from ISBER. She is a fellow of ASME and AIMBE and a National Blood Foundation Scholar.
REGenerative Medicine: Cryopreservation of Cells to Organs

Co-chairs: Mehmet Toner, Ph.D., Director, Center for Engineering in Medicine, Massachusetts General Hospital; Allison Hubel, Ph.D., Professor, Mechanical Engineering

Microfluidic Organoid Cultures for Personalized Treatment of Cancer

Authors: Neda Dadgar, Alan Gonzales, Gulnaz Stybayeva, Wen Wee Ma*, Mojun Zhu*, Alexander Revzin, Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN
*Department of Oncology, Mayo Clinic, Rochester, MN

There is increasing interest in utilizing cancer tissue from biopsies as patient surrogates for personalizing treatment of cancer. In this approach, biopsies are typically digested and resultant cancer cells are cultured on Matrigelin media promoting organoid formation. However, a typical biopsy is a sliver of tissue a few mm long with as few as 100,000 cells. This amount of cellular material is insufficient for testing multiple concentrations or combinations of anti-cancer drugs. This motivates the use of microfluidic cultures which allow one to minimize the amount of cellular material needed for identifying optimal therapy regimen. One issue experienced by us and others is the difficulty of cryopreserving functional and viable organoids from primary patient tumors.

Alexander Revzin, Ph.D.
Professor of Biomedical Engineering, Mayo Clinic

Alexander Revzin received B.S. degree from Wayne State University and Ph.D. Texas A&M University, both in chemical engineering. Subsequently, he spent two years as a postdoctoral fellow in Harvard Medical School/ MGH. Revzin joined Department of Biomedical Engineering at University of California, Davis in 2004 and rose to the rank of Professor. He moved to Mayo Clinic in October 2016 where he is a Professor of Biomedical Engineering. Revzin also served as an NSF program director overseeing the area of nano-biosensing. His research interests are at the intersection of cell/tissue engineering, biosensors and microfabrication.
CELL, TISSUE, AND ORGAN CRYOPRESERVATION—COMMON APPROACHES AND UNIQUE CHALLENGES

A perpetual problem with organ and tissue transplantation is the imbalance of supply and demand for organs. This challenge is exacerbated by the relatively short window of time in which an organ can survive outside of the donor’s body before transplantation. Cryopreservation, or storage of organs and tissues at ultralow temperatures (-140°C), has the potential to essentially stop biological time. Previous attempts at organ cryopreservation have failed due to cellular and structural disruption caused by ice crystal formation. One promising approach that overcomes the limitations of conventional strategies for cryopreservation is vitrification—that is, cooling organs so quickly that they cannot undergo the phase transition from liquid to solid ice. In this manner, the organ enters a stable glass-like cryogenic state, wherein viable storage is theoretically indefinite. The main challenge, however, is rewarming these organs without ice formation as the temperature rises. The required rapid heating rates (>50°C/min for many vitrification solutions) are significant engineering barriers in larger system volumes (i.e. > 3mL). In addition, temperature non-uniformity during rewarming produces thermal stress that can cause cracking within the biomaterial. Hence, speed and uniformity of warming are essential. We have developed a novel heating approach termed “nanowarming” that achieves both objectives. Tiny iron oxide nanoparticles are perfused with the vitrification solution throughout the vasculature of the organ. The organ can then be vitrified by cooling (an existing technology), but also rewarmed as needed by placing it in a radiofrequency coil that creates magnetic fields that induce heating within the iron oxide nanoparticles and therefore from within the organ. We have shown that this approach can rewarmed vitrified organs in animal model systems (rat and rabbit) with physical success and organ function. Each organ, and other biologic specimens, has common features and unique challenges that must be overcome. This review summarizes some of those features, and points out the directions necessary to enable indefinite “banking” of cells, tissues, and organs prior to transplant or other biomedical applications.
Globally aquatic species are under siege by local degradation of their habitats and global stressors. Each person within this symposium will highlight a different aquatic group and how they are trying to solve the problem of species conservation with cryopreservation. Here we will focus on the marine realm on coral reefs.

**Threat:** Approximately 50% of all coral reefs have disappeared in the last century. Within the last 50 years, local stressors such as predation from Crown of Thorn Starfish and nutrient pollution have severely impacted the Great Barrier Reef and Florida Keys National Sanctuary. Within the past 20 years, increased emissions of greenhouse gases have created warmer air and water temperatures, causing deadly local and global bleaching events. In the 1998 global bleaching event alone, 15% of the world’s coral died.

**Status of Applications or Conservation Through Cryopreservation:** Today, coral reefs are being conserved through various types of cryopreservation. Sperm cryopreservation has secured the diversity of 37 coral species worldwide. These resources have been used in Assisted Gene Flow to create new types of coral that are currently being tested for resiliency. Additionally, with the advent of ultra-rapid laser-warming, coral larvae from three species have been successfully cryopreserved and rewarmed.

**Future Directions:** Most cryopreservation of coral depends on successful reproduction. However, this is being impacted by bleaching events which degrade the quality of the gametes so that cryopreservation is less successful. Because of this, we are examining freezing coral fragments. This would allow cryopreservation many days of the year instead of the few weeks per year available during spawning events, but would produce clonal offspring instead of sexually assorted offspring. As we try to increase the size of our coral fragments, the engineering challenges become ever greater, due to the need to warm faster. Today, we are looking toward a variety of innovative tools that include Ice Recrystallization Inhibitors, ultra-rapid laser warming, radio frequency warming and potentially isochoric freezing processes. These engineering advances are critical to the successful cryopreservation that will allow us to secure the biodiversity and genetic diversity of many of the 1,000 species of corals on reefs around the world.
Zebrafish embryos can attain the cryogenic state by microinjection of cryoprotectants followed by rapid cooling, but the massive size of the embryo has consistently led to failure during the slower convective warming rates. Here we address this zebrafish cryopreservation problem by using gold nanorods (GNRs) to assist in the warming process. Specifically, we micro-injected the cryoprotectants and GNR into zebrafish embryos and cooled them at a rate of 90,000 °C/min in liquid nitrogen. Earlier, we demonstrated the ability to unfreeze the zebrafish rapidly \((1.4 \times 10^7 \degree C/min)\) by irradiating the sample with a 1064 nm laser pulse for 1 ms due to the excitation of GNRs. This rapid warming process led to the outrunning of ice formation, which can damage the embryos. The results presented here continue to demonstrate viable embryos with consistent structure at 1 hr (41%), and continuing development at 3 hr (22%), movement at 24 hr (11%) and hatching at 48hr (9%) post-warming. We now present new data that shows larval fish swimming at Day 5 (3%). In addition, we optimize various experimental parameters like injection volume, development stage and chorion removal post warming to improve the overall survival rate of this technique. Finally, two laser warmed fish were grown to adulthood and spawned to give embryos with over 90% survival. This nanoparticle-based warming process can be applied to storage of fish germplasm, and with proper modification, it can be potentially used for other vertebrate embryos.
CRYOPRESERVATION AND LASER NANOWARMING OF AQUATIC SEED FOR CONSERVATION AND AQUACULTURE
Authors: Kieran Smith, Kanav Khosla, Guebum Han, Tim Humphrey, Michael McAlpine, John Bischof

Genetic banking of plant cells, tissues, seeds, and mammalian embryos is common practice in agriculture to ensure important genetic lines are not lost due to disease outbreak or environmental catastrophe. However, genetic banking of aquatic embryos and larvae within the aquaculture industry is nonexistent to date, and the investments made toward genetic improvements are susceptible to catastrophic loss without a proper seed banking product. The share of aquaculture reproduction in total value chain output is quite small when compared to the shares of reproduction in other farming industries. For example, the cost of genetics constitutes 16% in corn farming as compared to just 1.5% in aquaculture. The relatively large size (0.2 – 5.0 mm) of aquatic ova and embryos and high yolk content is currently a barrier to the formation of an aquaculture seed storage product. Recent advancements in rapid cooling for storage at liquid nitrogen temperatures (-196°C) and ultra-rapid laser rewarming (>10⁷ °C/min) have led to major breakthroughs in cryopreservation technologies. These advancements have enabled successful cryopreservation of an important aquaculture species *Litopenaeus vannamei*, or Pacific white shrimp. The current best use protocol results in 89% resumption of movement and 55% active swimming of nauplius larvae. Optimization of low molarity (<3.0 M) CPA loading and unloading, vitrification, and ultra-rapid laser warming procedures are necessary to ensure long term survival and reproductive success of cryopreserved larvae. Multiple technologies (microfluidics, 3D printing, ultra-rapid laser warming) are being adapted to implement a genetic banking product to allow breeding facilities to “cryobank” thousands of seeds from family lines developed over years of *L. vannamei* selective breeding programs. These technologies can be adapted for the conservation of threatened or endangered aquatic organisms and protect genetic resources vital to the commercial aquaculture industry and food security domestically and abroad.
CRYOPRESERVATION IN THE CONTEXT OF EMBRYOGENESIS AND ANIMAL EVOLUTION

Evolution and embryogenesis are both natural processes that transform very simple forms of life into highly complex biological entities. The first multicellular animals, or metazoa, appear in the fossil record over 600 million years ago. These ancestral metazoans were relatively simple and more reminiscent of protozoan colonies with quasi-independent cells rather than what most would now recognize as animals. Through time, these ancestral metazoans gave rise to over 30 phyla of more highly developed animals in which cells cooperate to form tightly coordinated tissues and organs. Within each generation, embryogenesis transforms a relatively amorphous fertilized egg into highly-complex, multi-billion celled animals. Over hundreds-of-thousands of generations, minor changes in embryogenesis have resulted in novel animal forms and consequently given rise to the diversity of animal life present today. In this presentation, several topics will be discussed including how some of the intrinsic properties of embryogenesis may influence the development of successful embryonic cryopreservation techniques as well as the potential opportunities that embryonic cryopreservation may afford several scientific fields such as conservation biology, medical genetics, and sustainable food production.

Alexander Primus is an Assistant Professor in Fish Health at the University of Minnesota’s College of Veterinary Medicine. In this role, he provides support to local, regional, and international stakeholders in the aquaculture industry through a mixture of research, diagnostics, extension, and teaching. With over 15 years of professional experience in fish health management, veterinary diagnostics, fish vaccine R&D, and biomedical research, he is dedicated to the promotion of sustainable aquaculture practices and the development of innovative fish health management strategies.
AQUATIC SPECIES CRYOPRESERVATION

Co-chairs: Mary Hagedorn, Ph.D., Research Biologist, Smithsonian’s National Zoo & Conservation Biology Institute; John Bischof, Ph.D., Director, IEM

DIRECTING SUBZERO TOLERANCE TO TEMPERATURE SENSITIVE ZEBRAFISH

Authors: Luciana Da Silveira Cavalcante1,2; Reinier J. de Vries1,2; Juan Manuel González-Rosa3; David M. Langenau4; Korkut Uygun1,2; Mehmet Toner1,2; Shannon N. Tessier1,2,*

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The ability to preserve various types of aquatic life has far reaching implications for aquaculture, conservation of biodiversity, and scientific research laboratories. For example, cryopreservation of genetic material, sperm, eggs, and embryos could simplify broodstock management, facilitate breeding programs, secure a method to re-establish populations of threatened species, and enable the dissemination of important strains of laboratory species. For some ectotherms in nature, temperature variations beyond their tolerance limits can have deleterious consequences. Yet, numerous vertebrate lineages colonized colder environments and overcame the selection pressures of life at subzero temperatures. Survival in these cold-hardy organisms below 0°C depends on either freeze avoidance or freeze tolerance. In this work, we identify methods and agents in zebrafish that confer freeze tolerance or freeze avoidance at high subzero temperatures (between -10 and -20°C). Using zebrafish larvae, we screened for cryoprotectant agents which preserve heart rate, circulation, and morphology after subzero preservation. As a result of these screens, we identified promising cocktails which restored heartbeat in 82% of larvae immediately post-recovery. Subsequently, optimal cocktails from larval screens were tested on isolated adult zebrafish hearts that were cooled to -10°C and held for up to 24 hours. After rewarming, the metabolic rate of adult hearts stored for 24 h was similar to time-matched controls (0.213 ± 0.047 and 0.275 ± 0.060, respectively, p = 0.200). In summary, we present data to illustrate our efforts in converting a naturally intolerant zebrafish to one which can survive at subzero temperatures. These efforts in high subzero preservation may inform other cryopreservation protocols for diverse aquatic species.

Shannon Tessier, Ph.D.
Instructor, Massachusetts General Hospital and Harvard Medical School

Shannon Tessier, PhD, received her MSc and PhD in molecular biology and biochemistry from Carleton University in 2010 and 2014, respectively. She applied a wide range of molecular and cell biology approaches aimed at understanding the molecular mechanisms which support natural suspended animation. As a postdoctoral fellow, Dr. Tessier expanded her skillset into the field of biomedical engineering by joining the Center for Engineering in Medicine in 2014. Here, she applied her expertise in low temperature biology to develop new methods for whole blood stabilization to facilitate liquid biopsies for diagnostics. Currently, as junior faculty within the Department of Surgery at MGH, she is working on synergistic projects that incorporate experimental biology, regenerative medicine, and organ reengineering approaches to enhance preservation and address the organ shortage.