2024 AFRL Biotech Days Summit

Program

27-28 February 2024

Wright State University Student Union, Dayton, Ohio

Agenda Outline

TUESDAY, 27 FEB – DAY 1 (VIRTUAL MEETING LINK)

Morning Agenda

Time (EST)	Session	Presenter(s)
0700-0830	**Continental Breakfast**	
0730-0800	**Badge Pick-Up & Poster Set-Up**	
0830-0845	**Opening Remarks** Biotech Community of Practice	Dr. Seth Faith
0845-0945	**Key Note Session:** Regional Collaboration for Global Impact: Ohio's Biotech Imperative	Dr. Michael Triplett
0945-1015	Fireside Chat: Perspective on Biotech Across the DOD	Dr. Katherine Sixt, Dr. Tanisha Hammill, Dr. Jean-Paul Chretien
1015-1030	**Coffee Break**	

Symposium: Biomedical | Col (Dr.) Scott Sonnek, Dr. Christina Bowman

Time (EST)	Session	Presenter(s)
1030-1050	En Route Care Research Overview	Ms. Tamara Averett-Brauer
1050-1110	Infectious Disease Surveillance Initiatives at the Defense Centers for Public Health	Ms. Genny Cook
1110-1130	Extracorporeal Support as an Essential Aspect of Critical Care	Maj (Dr.) Elizabeth Powell
1130-1150	Oxygen Conservation in Resource Constrained Environments	Dr. Chris Blakeman
1150-1215	**Inter-Panel Discussion**	
1215-1330	**Lunch**	

Afternoon Agenda (Virtual meeting link)

Symposium: Biomanufacturing | Chair: Dr. Nicholas Wilson

Time (EST)	Session	Presenter(s)
1330-1350	Feedstock-independent REE Bioextraction to Increase Resiliency for DoD	Dr. Chia Suei-Hung & Dr. Kara Martin
1350-1410	Optimization, Testing and Scale-Up of Ready to Spray Biocement	Dr. Maneesh Gupta & Dr. Rhett Martineau
1410-1430	Investigation and Application of Natural Flying Systems in AFRL/RW	Dr. Nicholas Rummelt
1430-1450	BioMADE's Impact on Defense Biomanufacturing	Dr. Angela Campo
1450-1515	**Inter-Panel Discussion**	
1515-1535	Tri-service Biotechnology Resilient Supply Chain Program (T-BRSC) Overview	Dr. Nancy Kelley-Loughnane
1530-1540	OUSD Acknowledgments	Dr. Katherine Sixt
1540 - 1550	**Transition to Poster Session**	
1550 - 1720	**Poster Session**	

Evening Agenda

1800-2000	**No Host Social @ On Par Entertainment** 4464 Indian Ripple Road, Dayton, OH 45440

WEDNESDAY, 28 FEB – DAY 2 (VIRTUAL MEETING LINK)

Morning Agenda

Time (EST)	Session	Presenter(s)
0730-0830	**Continental Breakfast**	
0800-0830	**Badge Pick-Up**	
0830-0840	**Welcome Back Remarks**	Dr. Timothy Bunning
0840-0940	**Key Note Session:** The Future of Computing at AFRL	Ms. Alexis Bonnell
0940-1000	Biotechnology Investments and Transitions	Dr. Peter Emanuel
1000-1015	**Coffee Break**	

Symposium: Human Performance| Chair: Dr. Nicole Ray, Dr. William Aue

Time (EST)	Session	Presenter(s)
1015-1035	Next Generation Sensing and Assessment Technologies, Isn't Wearables All We Need?	Dr. Jorge Chavez Benavides
1035-1055	Navigating Team Dynamics: Multivariate Insights into Collaborative UAV Control and Performance	Dr. Michael Tolston
1055-1115	Development, Assessment, and Transition of Wearable Sensors and Digital Data Management: A Look Back at 10 Years of STRONG Lab & its Position for the Future	Dr. Adam Strang
1115-1135	The Two-way Interaction of Travelers' Diarrhea and the Gut Microbiome	Dr. Zachary Liechty
1135-1200	**Inter-Panel Discussion**	
1200-1315	**Lunch & Poster Breakdown**	

Afternoon Agenda (Virtual meeting link)

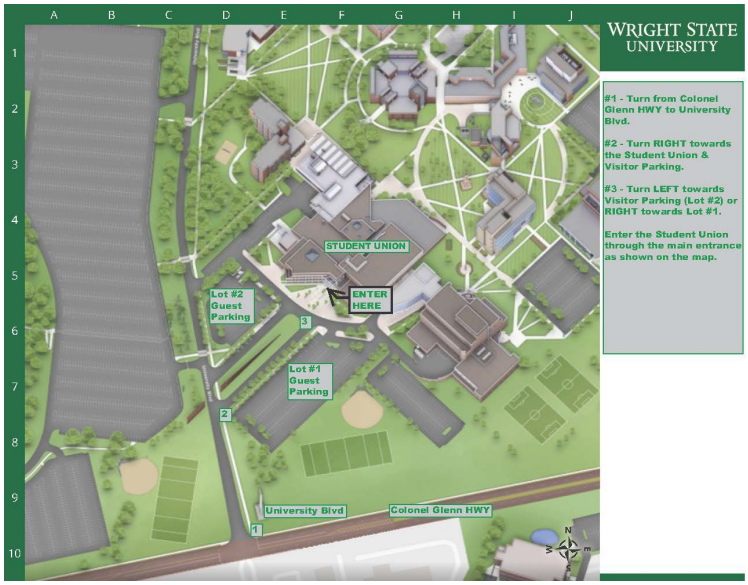
Symposium: Materials | Chair: Dr. Patrick Dennis

Time (EST)	Session	Presenter(s)
1315-1330	Bioprincipic Lessons from Invertebrates	Dr. Laura Bagge
1330-1345	Biologically-inspired Multifunctional Architected Materials	Prof. David Kisailus
1345-1400	Protein-mediated Polyphosphate Synthesis and Templating of Nanostructured Biocomposites	Dr. Peter Mirau
1400-1415	Iridescent Biofilms of Bacteria: Versatile Platforms for Functional Materials	Dr. Claretta Sullivan
1415-1430	Advances in Protein-based Biomaterials for DOD Application	Dr. Sanaz Farajollahi
1430-1500	**Inter-Panel Discussion**	
1500-1515	**Coffee Break**	
1515-1535	ARPA-H: The Mission	Dr. Andrew Kilianski
	AFOSR Tech Talks	Dr. Bennett Ibey
1535-1555	Materials Discovery through Exploration of Biomolecular Space	Dr. Rein Ulijn
1555-1610	Synthetic Mucus Biomaterials	Dr. Adam Braunschweig

Meeting Location and Parking

IN-PERSON MEETING LOCATION: Wright State University Student Union

The Student Union is located near the intersection of University Boulevard and Colonel Glenn Highway in Dayton, Ohio. See map below for parking directions.



ZOOM MEETING LOCATION:

Topic: 2024 AFRL Biotech Days Summit
Time: Feb 26, 2024 02:00 PM Eastern Time (US and Canada)
Feb 27, 2024 07:00 AM
Feb 28, 2024 07:00 AM

Join ZoomGov Meeting
https://uesincdayton.zoomgov.com/j/1607755087?pwd=Ti94N0pyajk4NytHSzU4Wmg5b1czdz09

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Symposium: Biomedical (Oral Presenters)

EN ROUTE CARE RESEARCH OVERVIEW

Authors:
Tamara Averett-Brauer, PhD(c), MN, RN, Senior Health Scientist/Nurse Researcher
Air & Space Biosciences Division, Enroute Care Section (RHBAM)
Human Effectiveness Directorate, 711th Human Performance Wing, AFRL

DESCRIPTION: This session presents an overview of the 711th Human Performance Wing En Route Care Research portfolio with an example of research studies in progress.
DISCUSSION: The great power competition and future peer/near-peer engagements present the risk for overwhelming casualties with severely constrained or limited ability to evacuate. The role of En Route Care Research Product Area is to develop and transition aeromedical evacuation (AE) and en route care (ERC) science and technology into knowledge and material products that promote the recovery and return to duty of injured or ill service members, from point of injury back to definitive care. Three lines of effort capture the portfolio work: 1. **Autonomous Care for Patient Movement** that seeks to Define, develop, and deliver new paradigms for providing AE care without human clinical interaction; 2. **Optimization of Patient Movement** that will extend ERC care providers through modeling and simulation that uncovers priorities for improved care models, tactics, teaming, training and equipment; and 3. **Ground Medical Operations in Agile Combat Employment** that will Inform and improve operational triage, combat casualty care, and expeditionary medicine in austere/prolonged care. “Future operational requirements call for clinically astute and operationally sound flyers. Therefore, it is **imperative to develop AE experts** with a breadth and depth of knowledge of the en-route care (ERC) system” *(Aeromedical Evacuation Flight Nurse Career Guide, 2023).* Addressing this challenge, a current study is presented: US Air Force Flight Nursing Expertise: A Qualitative Exploratory Descriptive study. Implications will be discussed for research and translation in education/training, practice, and policy.

INFECTIOUS DISEASE SURVEILLANCE INITIATIVES AT THE DEFENSE CENTERS FOR PUBLIC HEALTH

Authors:
Dr. Richard Agans

Force health protection and personnel readiness are crucial components to mission success within the DoD. While these topics cover broad areas, chief among them may be our ability to identify, track, and treat infectious diseases at unit/population scales. Respiratory infections can lead to 95,000 lost duty days encompassing 600,00 active-duty members each year. Similarly, 30% of active-duty members who spend >1 month traveling experience debilitating gastrointestinal distress and are incapable of carrying out duties and activities. The Defense Centers for Health Dayton (DCPH-D) is involved in a number of efforts aimed at surveilling relevant populations and environments for

infectious disease causing agents, characterizing threats within and across populations, and screening for potential countermeasures. DCPH-D utilizes a number of biotech driven approaches to accomplish its mission, including automated sample prep, next-generation sequencing, molecular detection via qPCR and mass-spec, classical virology and microbiology, and support from in-house bioinformatics enclaves. DCPH-D also maintains one of the few high containment BSL-3 laboratories within the DoD. From being an active leader in the DoD influenza surveillance, informing on vaccine effectiveness and targets for seasonal vaccines, to surveilling wastewater, soil, dust, and built surfaces for pathogens, to development of designer microbes for immune fortification, DCPH-D is a leader within the Defense Health Agency and DoD for ensuring warfighter health and readiness are maintained across geographies and missions.

EXTRACORPOREAL SUPPORT AS AN ESSENTIAL ASPECT OF CRITICAL CARE

Authors:
Maj (Dr.) Elizabeth K. Powell, MD

Extracorporeal support has evolved extensively over the years. Clinician comfort has increased and innovations in technology and applications have improved patient outcomes in and out of the hospital. Use for the acute stabilization and resuscitation of trauma patients has also improved outcomes. Future investment in training, technology, and novel applications will make extracorporeal support more readily available for injured warfighters.

OXYGEN CONSERVATION IN RESOURCE CONSTRAINED ENVIRONMENTS

Authors:
Chris Blakeman, Richard Branson, Michael Goodman

Oxygen is a finite resource in pre-hospital and military medicine. During COVID-19, oxygen shortages occurred around the world including the US. Oxygen conservation can be accomplished through simple behavior change or via technologic solutions. Each has a range of complexity and potential risks.

A simple behavior change such as using normoxic oxygen saturation via pulse oximetry (SpO2) targets can markedly improve oxygen conservation. Choosing a target SpO2 of 92-96% and avoiding hyperoxia can result in a savings of oxygen from 3-10 fold. This includes low flow oxygen via nasal cannula and mechanical ventilation.

Closed loop control of inspired oxygen (low flow or during mechanical ventilation) has been shown to reduce oxygen usage by 30-50% but requires FDA approval. Use of an oxygen concentrator allows oxygen delivery as long as electricity is available. Oxygen flow is directly related to concentrator size and weight. Portable concentrators are typically limited to 3-4 lpm and delivered fraction of inspired oxygen (FiO2) is inversely proportional to minute ventilation (respiratory rate x tidal volume). Combining a concentrator with closed loop control of oxygen combines advantages. One limitation of concentrator use is declining efficiency following prolonged storage or exposure to moisture.

Rebreathing systems can also conserve oxygen as the exhaled oxygen concentration is typically 2-3% less than inspired oxygen. Rebreathing can be done with a face mask (must be an airtight seal at the face) or with mechanical ventilation (not FDA approved) but is commonly used during anesthetic procedures. Routing exhaled gases from the expiratory limb of the ventilator to the inlet of the compressor or turbine requires additional safety measures. The impact of exhaled moisture and

potential contamination of the ventilator are risks. Rebreathing systems can allow FiO2 < 21% if oxygen source is loss. Safety would require monitoring of inspired O2 and CO2 (in case of absorbent failure) to prevent both hypoxemia and hypercarbia.
Oxygen conservation requires education and training of caregivers to understand application and safety.

Disclosure Statement:

The views, opinions, and/or findings contained in this journal article are those of the author and should not be interpreted as representing the official views or policies, either expressed or implied, of the Air Force Research Laboratory or the Department of Defense.

Symposium: Biomedical (Poster Presenters)

DEVELOPMENT OF A MOBILE-APPLICATION-BASED DIAGNOSTIC TEST FOR POST-TRAUMATIC STRESS DISORDER

Tony Dutcher, Veronica Choi, Alondra Chaire, Caitlin Limoncello, and Charles B. Nemeroff

Existing methods for Post-Traumatic Stress Disorder (PTSD) diagnosis and severity measurement rely heavily on patient self-report. These methods are generally effective but can be time-consuming, require specialized training, and rely heavily on the patient's willingness to disclose trauma and discuss their symptoms openly. However, there are numerous physiological and oculomotor signals associated with PTSD, including changes in heart-rate-derived metrics, gaze patterns, and pupil dynamics. Senseye (a Digital Health company) has developed computer vision techniques deployed using a mobile smartphone application to measure physiological and oculomotor responses from video of an individual's face. In this study, Senseye assessed the feasibility of using this technology to detect differences in oculomotor and heart-rate-derived metrics between PTSD patients and controls. Data was collected from 81 individuals, 32 PTSD+, and 49 controls. All participants completed a structured clinical interview, a variety of self-report measures, and the Senseye video scan. Our initial results show we can statistically discriminate PTSD status from healthy controls across a variety of physiological and oculomotor responses (p *values<0.01*). While statistical differences is a critical first step, we discuss and outline the necessary next steps to generate a diagnostic algorithm.

PURIFICATION AND PRODUCTION OF THE APOHEMOGLOBIN-HAPTOGLOBIN COMPLEX FOR THE TREATMENT OF HEMOLYTIC CONDITIONS

Quintin O'Boyle, Shuwei Lu, and Dr. Andre Palmer

Red blood cell (RBC) lysis (i.e., hemolysis) can occur in many different clinical scenarios including diseases such as sickle cell disease and malaria; as well, post transfusion of RBC or whole blood units. Hemolysis releases cell-free hemoglobin (Hb) into the extracellular space causing a cascade of harmful side-effects including vasoconstriction, systemic hypertension, inflammation, and tissue damage. These side-effects stem from the heme groups in Hb. In the blood, the naturally occurring plasma protein haptoglobin (Hp) binds to and neutralizes the toxic side-effects of Hb. In our lab, we have developed a process for purifying haptoglobin Hp) from Cohn fraction IV using tangential flow

filtration (TFF). Additionally, we have also developed a process to purify apohemoglobin (apoHb) via TFF, which is capable of scavenging cell-free heme. Thus, apoHb can function as a heme scavenger, similar to the plasma protein hemopexin (Hpx), whose function is to bind to and neutralize cell-free heme. We have demonstrated that Hp and apoHb can bind together to form the apoHb-Hp complex. This complex has the dual ability to bind Hb and heme. We show *in vitro* that the complex can bind to Hb and heme. Taken together, the complex could be used to treat the toxic side-effects associated with hemolysis.

POLYMERIZED HUMAN SERUM ALBUMIN AS A PLASMA SUBSTITUTE

Authors:
Andre F. Palmer[1], Qi Wang[1], Catalina Messmer[2], Ozlem Yalcin[3], and Pedro Cabrales[3]*
Affiliations:
[1]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, Ohio 43210
[2]Department of Otorhinolaryngology, Head and Neck Surgery, University of Munich, Munich, Germany
[3]Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093
**To whom correspondence should be addressed: E-mail: palmer.351@osu.edu*

Plasma substitutes (PSs), although they lack O_2 carrying capacity, are an important class of transfusion solution that can maintain blood volume. However, conventional PSs are often limited by undesirable side effects, such as RBC aggregation and nephrotoxicity (dextrans), coagulation disturbances (hydroxyethyl starches) and limited intravascular retention (albumin). Notwithstanding this minor limitation, albumin is considered a near optimal PS, whose unique molecular size, shape and electrical charge prevents vascular extravasation into most tissues. Despite its natural prevalence in the bloodstream, human serum albumin (HSA) can increase the risk of mortality when administered to patients with increased vascular permeability (i.e., patients suffering from burns, septic shock, ischemia-reperfusion injury and endothelial dysfunction). This occurs in injured vascular endothelium with increased capillary permeability resulting from physical damage, inflammation, neutrophils or endothelial swelling. We have demonstrated that the deleterious effects of HSA extravasation can be decreased/eliminated by polymerizing HSA (PolyHSA), in order to increase its molecular size, prevent extravasation and increase intravascular retention, while simultaneously decreasing its colloid osmotic pressure and increasing its solution viscosity. Our work on PolyHSA has demonstrated its ability to increase plasma viscosity, which induces mechanotransduction of the endothelium and elicits vasodilation and increased tissue perfusion.

PILOT SCALE PRODUCTION AND CHARACTERIZATION OF NEXT GENERATION HIGH MOLECULAR WEIGHT AND TENSE QUATERNARY STATE POLYMERIZED HUMAN HEMOGLOBIN.

Authors:
Clayton T. Cuddington, Savannah R. Wolfe, Donald A. Belcher, Megan Allyn, Alisyn Greenfield, Xiangming Gu, Richard Hickey, Shuwei Lu, Tanmay Salvi, Andre F. Palmer.

Polymerized human hemoglobin (PolyhHb) is being studied as a possible red blood cell (RBC) substitute for use in scenarios where blood is not available. While the oxygen (O_2) carrying capacity of PolyhHb makes it appealing as an O_2 therapeutic, the commercial PolyhHb PolyHeme® (Northfield Laboratories Inc.) was never approved for clinical use due to the presence of large quantities of low molecular weight (LMW) polymeric hemoglobin (Hb) species (<500 kDa), which have been shown to elicit vasoconstriction, systemic hypertension, and oxidative tissue injury in vivo. Previous bench-top scale studies in our lab demonstrated the ability to synthesize and purify PolyhHb using a two-stage tangential flow filtration purification process to remove almost all undesirable Hb species (>0.2 µm and <500 kDa) in the material, to create a product that should be safer for transfusion. Therefore, to enable future large animal studies and eventual human clinical trials, PolyhHb synthesis and purification processes need to be scaled up to the pilot scale. Hence in this study, we describe the pilot scale synthesis and purification of PolyhHb. Characterization of pilot scale PolyhHb showed that PolyhHb could be successfully produced to yield biophysical properties conducive for its use as an RBC substitute. Size exclusion high performance liquid chromatography showed that pilot scale PolyhHb yielded a high molecular weight Hb polymer containing a small percentage of LMW Hb species (<500 kDa). Additionally, the auto-oxidation rate of pilot scale PolyhHb was even lower than that of previous generations of PolyhHb. Taken together, these results demonstrate that PolyhHb has the ability to be seamlessly manufactured at the pilot scale to enable future large animal studies and clinical trials.

SENSOR PROBE DESIGN TO MEASURE FIVE SKIN PROPERTIES FOR FROSTBITE INJURY ASSESSMENTS

Authors:
Daniel Sim, Zachary E. Brooks, and Steve S. Kim

Frostbite is one of the significant traumatic injuries in a cold environment and significantly affects Warfighter performance and missions. Therefore, medical assessment/treatment protocols and equipment for traumatic frostbite care are necessary to minimize the loss of military personnel in the operational field. A skin analyzer is a non-invasive skin property measuring device in contact with the skin surface. It can analyze skin conditions for early identification of skin injury, which can be used as a toolset to mitigate cold weather impacts on the pathophysiology of traumatic injuries. The more skin properties we measure, the more comprehensive and in-depth understanding of the skin we can obtain than the single property-based analysis. Previously, commercially available skin analyzers utilized a series of multiple probes and measurement protocols to quantitate multiple skin properties. Additionally, using multiple probes, and thus multiple potential sampling locations, has increased measurement uncertainty. Here, we present a skin-analyzing prototype with five sensor elements integrated into a single-device structure. The present prototype can rapidly and accurately measure critical multiple skin properties of transepidermal water loss (TEWL), temperature, conductance, hardness, and color. The presented prototype skin analyzer enables rapid and objective skin health assessment essential for various military missions in extreme environments. In addition, the skin analyzer is transportable and easy to use once further testing and circuit/power integration work is complete. Thus, the final skin analyzer can be deployable within the operational area, field hospitals,

and/or en route, enabling medical providers to perform early and efficient medical interventions to augment care during all transport phases of combat-related injuries. This capability that rapidly and accurately assesses skin injuries enables expeditionary medical providers at the point of need to sustain Warfighter and unit lethality, appropriately allocate resources, and prevent unnecessary evacuations.

A MICROFLUIDIC BIOHYBRID SENSOR PLATFORM FOR VOLATILE ORGANIC COMPOUND DETECTION

Authors:
Elisabeth M. Steel[1,2], Zachary E. Brooks[1,2], Maegan Kornexl[1,2], Angela Dixon[3], Mark Willis[3], Steve S. Kim[1]
Affiliations:
[1] 711th Human Performance Wing, Air Force Research Laboratory (AFRL), Wright-Patterson Air Force Base, Dayton, OH, USA
[2] UES, Inc., 4401 Dayton-Xenia Rd, Dayton, OH, USA
[3] Department of Biology, Case Western Reserve University, 2080 Adelbert Road, Cleveland, OH, USA
Email: steve.kim.13@us.af.mil

Selective, real-time detection of volatile organic compounds (VOCs), whether present in the atmosphere or human breath, remains elusive using synthetic systems due to limitations in detection limits and specificity. The olfactory system naturally discriminates a multitude of VOCs over a range of concentrations making it attractive for integration into sensing devices. The insect antenna sensor is a cutting-edge biohybrid platform that detects VOCs at lower concentrations with faster response times than traditional electronic sensors. While the excised insect antenna enables integration into electronic platforms, its functional electrical activity is limited to two hours, which has prevented comprehensive characterization studies and field deployment.
We have extended antenna lifetime from 2h to 14 days by implementing a 3D printed microfluidic set-up that demonstrates bioelectrical responsiveness to VOCs. Media was perfused through a hydrogel cast in two parallel microfluidic channels supporting each end of a Manduca sexta antenna. The antenna spans a gap in the microfluidic device that serves as a VOC sampling chamber. A 32-channel silicon microelectrode array (MEA) was integrated into the supported antenna to collect olfactory sensory neuron (OSN) firing patterns. Electrophysiology recordings were conducted while the biohybrid sensor was exposed to an air stream with and without acetone, toluene, isopropyl alcohol, and hexane at 20 ppb and 20 ppm concentrations (0.450 L/min). Neuron firing rate histograms were passed through a custom artificial neural network to classify VOC identity, reaching 90% accuracy. Future work leveraging genetic engineering of olfactory receptor expression will enhance VOC selectivity and sensitivity of biohybrid sensors.

Symposium: Biomedical (Demo Presenters)

MULTIPLEXING BIOSENSOR BASED RAPID DIAGNOSTIC PLATFORM FOR FORCE HEALTH PROTECTION

Authors:
Dr. Joshua Sestak, Dr. Andrew Skaff, Sean McIntosh

The military veteran community is impacted by controlled substance abuse and post-traumatic stress disorder at a higher percentage than the non-veteran population. Before structural change can be made, there is a need to quantify the severity of the drug abuse or mental health problem and develop a roadmap to recovery that includes daily or weekly monitoring of related biomarkers. Saliva is being utilizes as a medium in which mental health or stress related biomarkers like cortisol, dopamine, or ACTH. The Veteran's Administration has funded university led research and is currently evaluating new diagnostic tools to provide greater care to military veterans[1]. However, there is still a capability gap between colorimetric lateral flow assay (pregnancy tests) style diagnostics and expensive lab equipment in processional laboratories. Portable, low-cost, easy to use electrochemical based rapid diagnostic systems are highly desired, particularly for triaging new patients at point of care facilities and observing patient dosage schedules. Since 2015, there have been several attempts to create electrochemical driven, 3D manufactured Lab On A Chip platforms at leading research institutions. The University of California - Santa Barbara DStat project and Whitesides Group Cheapstat[2] project at Harvard are two prominent examples. Additionally, electrochemical methods of screening for controlled substances or their related metabolites in saliva samples is one of the most cost viable methods of trace detection. Optical methods require larger power source and have non-specific binding effects that are hard to screen out and the cost of the lens and other materials in an optical detection method cannot easily be overcome. Colorimetric testing methods require sample consistency, have a low shelf life, and are prone to false negatives. Our electrochemical method, paired with powerful machine learning software has a very low "per test" cost and takes less than ten minutes to use by a trained technician. The value of diagnostic tests and tools in healthcare is indisputable, whether it be for human performance, monitoring of patient recovery, pandemic pathogen detection, or medical prescription adherence. While traditional diagnostic testing methods have progressed immensely in recent decades, there are still non-ideal aspects of common testing methods that prevent them from being more widely accessible. For example, they are commonly analog (i.e., not always a simple yes/no result), use specialized equipment and require trained personnel. These lab diagnostics are also expensive and typically done in centralized labs, which requires time for transport and/or multi-day analysis as well complex sample preparation steps. In other words, for as far as we've come in recent decades, we still do not have diagnostic capabilities for most applications of need that can support in-field testing that is rapid, rugged, fast, affordable, reliable, and requires minimal user training.

AUTOMATED DECISION SUPPORT FOR REMOTE HEALTH MONITORING

Authors:
Alyssa Tanaka, Ph.D., Jon Sussman Fort, Ph.D., and Joseph Cohn, Ph.D.

The support of medical personnel at the point of injury in austere or hostile environments is an ongoing challenge. Special Operations Forces (SoF) personnel will continue to operate in these combat settings, which are typified by contested logistics, prolonged field care needs, and a lack of organic healthcare assets. SoF medical personnel will need new diagnostic and treatment capabilities to effectively treat injured warfighters at Point of Injury (POI) that do not: (1) significantly increase their overall kit size, weight, or power needs
and (2) significantly detract from their ability to maintain situational awareness and decision advantage. Soar Technology LLC is currently developing an artificial intelligence/machine learning (AI/ML) medical support capability that addresses these challenges. This solution, the Special Forces Autonomous Vital Injury Observation and Rescue (SAVIOR), focuses on providing an isolated, injured warfighter with a capability to detect, diagnose, and communicate to medical support teams their health status and immediate medical requirements following a casualty event.
SAVIOR integrates into a mobile device with wearable sensors and an AI agent to continuously monitor patient physiologic status and survival supplies and send periodic updates to support teams at the Joint Personnel Recovery Command (JPRC). SAVIOR will provide an over-the-horizon link between the patient, medical support teams and command and provide detailed information for recovery and support. Specifically, SAVIOR will:

- Monitor patient vitals, resource availability and consumption, and environmental conditions.
- Provide diagnoses, as well as treatment and intervention recommendations.
- Communicate patient health and medical team supply status for medical consultation, prioritized resupply, support, and recovery.

SAVIOR builds upon SoarTech's experience in developing intelligent systems for autonomous care which includes domain-agnostic AI/ML applications that: leverage natural language processing for intuitive user interaction and effective speech understanding
require minimal training data and work with limited input data
quickly adapt to different domains and contexts
and integrate with other AI/ML tools and data capture capabilities like physiologic monitoring. Its current user community are special forces operators. SAVIOR is currently at TRL 5 and has been tested in relevant environments with support of the Navy and Air Force (Joint Exercise Tarpon Springs, 6/2023) and Coast Guard (Joint SERE Air Force Coast Guard Exercise 8/2023). Further development is under way to enhance system predictive capabilities across multiple patient states and to provide improved user interface software as part of a full Android Team Awareness Kit (ATAK) plug-in compatible with BATDOK (Figure 1).

SAVIOR is designed to monitor, collect and analyze physiologic measurements, integrating via Bluetooth with multiple monitoring and diagnostic devices, including the Human Systems Integration Physio Shirt and Garmin Tactix 7 watch, and aggregating and analyzing data to provide diagnosis and intervention recommendations. The system acts as an edge device, communicating via SATCOM using the Garmin inReach Mini 2 device to synch with other edge devices or a cloud-based system when available. As a software-driven solution, the entire SAVIOR System – device suite, AI/ML capabilities, and communications protocols - is extensible. SAVIOR's current application to SERE (Survival, Evasion, Resistance, Escape) operations and recovery of an isolated person is only an instance of its broader application.

Symposium: Biomanufacturing (Oral Presenters)

FEEDSTOCK-INDEPENDENT REE BIOEXTRACTION TO INCREASE RESILIENCY FOR DOD

Authors:
Chia-Suei Hung[†1], Kara Martin[†1], Emily Koerner[1,2], Josh Mancini[1,2], Martha Carter-Gerakines[1], Amy Breedon[1,2], Andrea Hoffman[1,3], Loryn Bowen[1,3], Adam Reed[1,3], Hao-Bo Guo[1,2], Selemon Bekele[1,2], Alexander Perminov[1,2], Baxter Huntington[1,2], Rajiv J. Berry[1], Nancy Kelley-Loughnane[1], Oscar Ruiz[1]
Affiliations:
[†]co-presenter
[1]Air Force Research Laboratory, Wright-Patterson AFB, OH, USA
[2]UES, Dayton, OH, USA
[3]University of Dayton Research Institute, Dayton, OH, USA

Rare earth elements (REE) are essential components of many advanced technologies from permanent magnets to DoD assets. However, harvesting REE in the US has been severely limited as current approaches for recovering and purifying individual REE are not environmentally friendly, are energy and solvent intensive, and cannot easily separate individual REE. To address these drawbacks and strengthen the domestic REE supply chain, we are focused on developing reliable biology-based approaches that can economically and effectively separate and recover individual REE from a diverse range of sources including mine tailings, e-waste, acid mine drainage, and contaminated groundwater. To separate REE, we are developing highly selective binding proteins and peptides for bio-separation through the application of a broad range of innovative approaches including directed mutagenesis, high throughput library screening, and computational modelling. We are examining the performance of different platforms to display engineered biomolecules. We are also engaging several small businesses involved in REE recovery to ensure future transition of our bioprocesses. These technologies will allow for the developments of greener REE extraction methods and ensure domestic REE supply-chain resiliency.
Key words: *Rare Earth Element; Bioextraction; Biotechnology; Synthetic Biology.*

OPTIMIZATION, TESTING AND SCALE-UP OF READY TO SPRAY BIOCEMENT

Authors:
Rhett Martineau, Maneesh Gupta, Michael S. Carter, Matthew J Tuttle, Brandon M. Bradow, Jessica Findsen, Eleanor K. Chase, Kristen A. Bruce

Biomineralization through microbially-induced calcium carbonate precipitation (MICP) is a process that has shaped Earth's geological features yet can also be harnessed as a green technology for civil and environmental engineering applications. Sporosarcina pasteurii is a soil bacterium that has emerged as the model organism for biocement production. We have developed and optimized a shelf-stable formulation of *S. pasteurii* that permit biocement production across scales, including field-based production using natural soil as a substrate. Through various field tests we have demonstrated that this formulation can be used to rapidly and reliably biomineralize calcium carbonate, leading to *in situ* biocement production and increased bearing capacity of the ground. Our results indicate that stabilized preparations of bacteria are a viable means by which biocement may be manufactured. This is advantageous as it can be produced at industrial scales and used as a ready-mix formula for the creation of biocement that is accessible to nontechnical professionals. Shelf-stable formulas such as this will make the process of biocement production easier, paving the way for more widespread adoption of biocement as a construction material.

INVESTIGATION AND APPLICATION OF NATURAL FLYING SYSTEMS IN AFRL / RW

Authors:
Nicholas Rummelt, Martin Wehling, Laura Bagge, Kristen Landmann

AFRL/RW studies flying biological systems, particularly insects, for inspiration for advancing sensor technology for autonomous guidance, navigation and control of airborne platforms. Our investigations include natural system sensors, and information extracting and processing 'algorithms,' leading to bioprincipic designs, with products intended for manufacturing
the focus is on insect vision systems and development of bioprincipic seekers at the component level and the seeker system level, compatible with autopilots and airframes. We will provide a high level overview of our laboratory environment and examples of products.

BIOMADE'S IMPACT ON DEFENSE BIOMANUFACTURING

Authors:
Angela Campo

Advances in bioindustrial manufacturing are poised to solve critical chemical supply chain risks to our national security. A wide array of critical chemicals in the defense supply chain are sourced from overseas and are part of a fragile global supply ecosystem. Catalyzed by the U.S. Department of Defense and charged with helping to develop and sustain a bioindustrial manufacturing ecosystem in the United States, public-private partnership BioMADE is funding dozens of projects that improve the efficiency at which biobased products can be developed and brought to market. This presentation will introduce example solutions that advance key technologies to provide critical chemicals to the defense industrial base and increase resilience against disruptions due to threats such as climate change. It will highlight efforts from BioMADE's diverse technical portfolio, methods to improve downstream processing to remove and purify a variety of industrial products and modeling product supply chains to increase efficiency and resiliency. By focusing on generalizable and precompetitive technologies, these efforts address the array of expensive one-off processes that have slowed industry growth. By providing improved unit operations, standardized processes, and efficiencies in process development, BioMADE's technical efforts help to reduce the costs of bringing a biobased product to market and especially the costs of production. When these costs are lowered, the number of critical chemicals that can be brought to market increases rapidly. Thus, these efforts are essential in reshoring manufacturing and securing a domestic supply chain for a wide range of products.

TRI-SERVICE BIOTECHNOLOGY FOR A RESILIENT SUPPLY CHAIN (T-BRSC) OVERVIEW

Authors:
Ben Wolfson, Steve Knott, Maneesh Gupta, Anthony Malanowski, Sandy Gibbons, Steve Maul, Nick Thomas, Nancy Kelley-Loughnane

The Tri-Service Biotechnology for a Resilient Supply Chain (T-BRSC) program is a five year, $330M

investment to utilize biomanufacturing, accelerate transition, and innovate and lead in biotechnology for defense. T-BRSC integrates fundamental and early applied research across four technology focus areas: Enhanced Capabilities, Reduced Logistics, Cost-Savings and Infrastructure Modernization. T-BRSC is represented by scientists and engineers from across the Military Services and Components, as well as their industry and academic partners, who are collaborating to establish a pipeline for advanced development and transition of biomanufactured products for defense use.

Symposium: Biomanufacturing (Poster Presenters)

DEVELOPMENT OF BIOCIDES FOR MITIGATION OF MICROBIAL FUEL CONTAMINATION

Authors:
Amanda L. Barry Schroeder[1], Adam M. Reed[1], Loryn L. Bowen[1], Osman Radwan[1], Oscar N. Ruiz[2], Andrea Hoffmann, Thusitha S. Gunasekera[3]
Affiliations:
1 University of Dayton Research Institute, Dayton, OH
2 RXEB, Biomaterials Branch, Photonic, Electronic & Soft Materials Division, Materials & Manufacturing Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH
3 RQTF, Fuels & Energy, Turbine Engine Division, Aerospace Systems Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH

POC Email: amanda.barry_schroeder.ctr@us.af.mil

Aerospace fuel contamination with bacterial and fungal microbial species represents a major challenge to the US Air Force and demands effective mitigation strategies. Biological control agents appear to be promising alternatives to chemical biocides. This project examined the antimicrobial activity of secreted metabolic products from fuel contaminating microbial strains as a means to inhibit growth of other competing microbial species of interest contained in fuel. In this study, we screened over 500 microbial fuel isolates for their potential antimicrobial action. As a result, we have identified *Pseudomonas protegens* (isolate #133) as an effective antimicrobial producer that can inhibit the fuel-based growth of a chosen set of four important microbial contaminants e.g., gram-positive bacterium *Gordonia sp.*; gram negative bacterium *Pseudomonas putida;* and fungal strains such as *Yarrowia lipolytica* and *Hormoconis resinae*.
Key Words: biocides, fuel contamination, *Pseudomonas protogens*.

BIOTECHNOLOGY EDUCATION AND WORKFORCE DEVELOPMENT AT UES

Author:
Amy Breedon, Stephaney Shanks, Lucas Beagle

At UES, we thrive by bringing the best and the brightest talent together to advance the future of science and technology. In order to solve tomorrow's scientific challenges and equip and empower the next generation, we need to invest in education and early workforce development today. Therefore, we support a variety of STEM education and outreach activities across our organization.

Here, we'll highlight various initiatives, including our work with the Nano-Bio Materials Consortium internship program and our recent Dayton bioManufacturing Awareness and Discovery Experience (DaytonMADE) camps for high school students.

RAPID BIOTECHNOLOGY PROTOTYPING CONSORTIUM

Authors:
Henry S. Gibbons[1,4], Rhett Martineau[2], Chia-Suei Hung[2], Oscar Ruiz[2], Gary Vora[3], Judson Hervey[3], Kelly Basi[4], Randy Hoffman[4,5] Nathan McDonald[4], Jessica Paradysz[4], Annie Crumbley[4], James Sumner[5], Randy Hughes[5], Ben Wolfson[1], Nancy Kelley-Loughnane[1,2]
Affiliations:
[1]Tri-Service Biotechnology for a Resilient Supply Chain Program Management Office
[2]Air Force Research Laboratory
[3]Naval Research Laboratory
[4]DEVCOM Chemical Biological Center
[5]Excet, a Precise Systems Company
[6]DEVCOM Army Research Laboratory
[*]Presenting author

The Rapid Biotechnology Prototyping Consortium (RBPC) represents the effort to modernize the biotechnology laboratory infrastructure and capabilities that is funded by the Triservice Biotechnology for Resilient Supply Chains Program. Consisting of research groups and subject matter experts at several DoD service laboratories across the United States, the RBPC is designed to employ best practices drawn from industry and academia to execute a complete biotechnology design-build-test-learn cycle from gene synthesis, strain engineering, process development, through to scale-up. Data sharing and technology transfer across the RBPC is facilitated by a robust, secure, and flexible digital backbone that will exploit the extensive resources of the High-Performance Computing Modernization Program to enable computation-intensive operations such as metabolic modeling. By employing "Begin with the End in Mind" and "Scale-down to Scale-up" systems engineering principles and implementing interdisciplinary integrated product teams for specific molecules or materials, the RBPC will compress the timeline of military-relevant biotechnology products from ideation to prototypes that incorporate biomanufactured materials.
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BIOTECHNOLOGICAL PRODUCTION OF PYOCHELIN SIDEROPHORE IN RARE EARTH ELEMENT ENRICHMENT

Authors:
Adam M. Reed[1], Amanda L. Barry Schroeder[1], Oscar N. Ruiz[2], Chia-Suei Hung[2], Andrea Hoffmann[1] and Kara L. Martin[3]
Affiliations:
University of Dayton Research Institute, Dayton, OH
RXEB, Biomaterials Branch, Photonic, Electronic & Soft Materials Division, Materials & Manufacturing Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH
RQTF, Fuels & Energy, Turbine Engine Division, Aerospace Systems Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH

POC Email: adam.reed.16.ctr@us.af.mil

Low-cost storage technologies and more efficient engines are critical for energy generation from renewable sources. Rare earth elements (REE) have been identified as resources needed for battery energy storage units, consumer electronics and electric vehicles. The harvest and recycling of rare elements has become of increasing demand for use in military and aerospace applications such as energy-saving lamps, lasers, radar, computer drives, electric motors, and as catalytic converters in industrial applications. This study is thought to develop a novel methodology that uses the ion chelator Pyochelin as an affinity compound for purification of lanthanide REEs such as Nd, La, Tb, Dy, Yb, Eu.

Key words: pyochelin, siderophore, rare earth elements.

A PROTEIN BIOMATERIALS PROTOTYPING FACILITY (PRO2FAC) AT THE AIR FORCE RESEARCH LABORATORY

Authors:
Kristi M. Singh, Patrick B. Dennis, Jackson D. Harris, Iman Eizadynejad, Rhett L. Martineau

To advance developing biomaterials through higher technology readiness levels (TRLs), it's important to be able to scale up material production beyond the Air Force Research Laboratory's historical capabilities. To this end, a pilot-scale fermentation facility is being built which expands current capabilities and supports DoD supply chain resiliency. The new facility design is currently in progress and will include upstream and downstream equipment necessary to support 100 L fermentations, with a particular emphasis on producing protein-based biomaterials. An overview of laboratory design, equipment selection, and proposed workflows, along with potential biomaterials projects, will be presented. The creation of a new protein production scale-up facility will pave the way for realizing real-world applications of biologically-based materials.

TRI-SERVICE BIOTECHNOLOGY FOR A RESILIENT SUPPLY CHAIN

Authors:
Ben Wolfson, Steve Knott, Maneesh Gupta, Anthony Malanowski, Sandy Gibbons, Steve Maul, Nick Thomas, Nancy Kelley-Loughnane

The Tri-Service Biotechnology for a Resilient Supply Chain (T-BRSC) program began in Fiscal Year 2022 with the goal of develop a tri-Service biotechnology pipeline for advanced development and transition of biomanufactured materials for defense supply chain resilience. T-BRSC brings together Joint Service partners to leverage significant advances made over the last decade in using microorganisms to produce highly specialized bio-based chemicals that can be used to manufacture a wide variety of materials of interest to the Department of Defense. The T-BRSC project portfolio is focused on Enhanced Capabilities, Reduced Logistics, Infrastructure Modernization, and Cost Savings, and is emphasizing the rapid prototyping of promising biotechnology research through partnerships with non-traditional commercial performers to facilitate entry of biomanufactured materials into acquisition Programs of Record and develop the Biotechnology Defense Industrial Base.

Symposium: Human Performance (Oral Presenters)

KEYNOTE: THE FUTURE OF COMPUTING AT AFRL

Authors:
Alexis Bonnell

Abstract not available at this time.

NEXT GENERATION SENSING AND ASSESSMENT TECHNOLOGIES, ISN'T WEARABLES ALL WE NEED?

Authors:
Jorge Chavez Benavides

Technologies to continuous measuring vitals (heart rate, heart rate variability, etc.) provide unprecedent insights into human physiology and opened the door to learning how our bodies respond to different physical and mental stressors. Continuous glucose monitoring devices (CGMDs) have revolutionized diabetes treatment, and the development of insulin pumps that can be connected to GCMDs promise to provide closed loop systems to manage diabetes. In this presentation we will discuss the new generation of wearable devices being developed to measure biomarkers in different biofluids (sweat, interstitial fluid, etc.). Finally, we will explore novel in-body technologies to monitor our body biochemistry from within in real-time and their potential impact in the DOD and AF mission.

NAVIGATING TEAM DYNAMICS: MULTIVARIATE INSIGHTS INTO COLLABORATIVE UAV CONTROL AND PERFORMANCE

Authors:
Dr. Michael Tolston

As the Air Force moves toward realizing Joint All Domain Command and Control (JADC2), there will be an increased need for robust data pipelines and metrics that enable efficient and effective distributed teaming. In particular, this means there will be a need for methods that can map multivariate sources of complex, non-stationary data to team performance outcomes. In the present work, we identify multivariate predictors of team coupling and performance in a collaborative UAV control task by evaluating non-stationary multiplex recurrence networks generated from time series data. Our analysis leverages advanced techniques, including tensorial decomposition of multilayer networks, to measure interactions between teammates in a simulated joint UAV control task and identifies three distinct phases of team interaction. Implications and future directions will be discussed.

DEVELOPMENT, ASSESSMENT AND TRANSITION OF WEARABLE SENSORS AND

DIGITAL DATA MANAGEMENT: A LOOK BACK AT 10 YEARS OF STRONG LAB & ITS POSITION FOR THE FUTURE

Authors:
Adam Strang

AFRL's STRONG lab develops, test and fields technologies for DoD's embedded human performance teams and programs. This presentation will describe STRONG's recent advances in the development and transition of DoD-approved data management and analytic platforms, review its progress for supporting a set of large-scale field and laboratory studies, and describe how the lab is positioning itself to support the development and transition of human performance technologies in future years.

THE TWO-WAY INTERACTIONS OF TRAVELERS' DIARRHEA AND THE GUT MICROBIOME

Authors:
Zachary Liechty[1], Arianna Baldwin[1], Blake Stamps[2], Adrienne Hatch-McChesney[3], J. Philip Karl[3], Michael Goodson[1]
Affiliations:
1 711th Human Performance Wing, Air Force Research Laboratory
2 Materials and Manufacturing Directorate, Air Force Research Laboratory
3 Military Nutrition Division, US Army Research Institute of Environmental Medicine

Personnel deployed to new environments are commonly exposed to pathogens which can result in impaired health and performance, reducing their effectiveness. The most frequently reported health issue amongst deployed personnel is diarrhea. Diarrhea can afflict over 50% of deployed personnel at some point during deployment, with the majority of those affected experiencing multiple episodes. While the probability of experiencing diarrhea can be somewhat attributed to lifestyle choices while deployed (i.e., handwashing, eating MREs over local economy, etc.), these lifestyle choices do not fully explain susceptibility to diarrhea inducing agents. Another factor that could influence diarrhea susceptibility is the composition of the deployed personnel's gut microbiome. Microbe-microbe interactions could inhibit or enhance the activity of a diarrhea-inducing pathogen, leading the deployed personnel to potentially harbor a "resistant" or "susceptible" microbiome. To investigate this possibility, we surveyed the gut microbiomes of personnel from 25th Combat Aviation Brigade (Wheeler Army Airfield, Hawaii) before and after the participation in Exercise Salaknib 2022, an approximately 2 month deployment to the Philippines. We additionally collected information regarding the dietary and lifestyle habits prior to and during deployment. We have found a distinct subject-dependent shift in the microbiome after deployment compared to before. Furthermore, some of the changes in relative abundance of specific ASVs (amplicon sequencing variants) were dependent on whether the host experienced diarrhea or not, suggesting diarrhea events could have long term specific impacts on microbial composition. Furthermore, several ASVs from the pre-deployment samples were found to be positively or negatively associated with experiencing diarrhea while deployed. Some of these ASVs could make promising targets to be used as a basis for prophylactic treatment in deployed personnel to reduce the incidence of diarrheal disease during and after deployment.
Disclaimer: Authors' views not official U.S. Air Force, Army or DoD policy.

Symposium: Human Performance (Poster Presenters)

DEVELOPMENT OF SEMI-SPECIFIC TRANSCRIPTION FACTOR ARRAYS FOR BIOSIGNATURE SENSING

Authors:
Kathryn Beabout, Svetlana Harbaugh, Simon d'Oelsnitz, Andrew Ellington, and Jorge L. Chávez

Preventing exhaustion and burnout is critical to maintaining safe and efficient operations in harsh environments. Insights into the status of personnel can be gained by tracking stress and fatigue biomarkers, such as cortisol and serotonin, which in turn can be used to implement safe and efficient workloads, routines, and practices. However, current technologies to measure biomarkers are impractical to implement in the field, as they require samples that are invasive to collect and assays that must be run in a laboratory. One challenge to developing a fieldable platform is a lack of recognition elements with high specificity and sensitivity towards each stress-related biomarker. To overcome this obstacle, we are working to engineer semi-specific transcription factors with affinity towards classes of biomarkers, such as steroids, catecholamines, and indolamines, rather than individual biomarkers. To achieve this, we are using a rapid selection approach to generate transcription factors in whole cells and their associated cell-free expression systems. To date, we have identified several promising variants with increased responses to target biomarkers. The variants will be coupled to an electrochemical output for multiplexed detection that mimics natural mammalian sensing mechanisms, such as the olfactory system, and allows for the detection of many complex chemical mixtures with relatively few receptors. Ultimately, this effort will lead to the development of a novel point-of-use sensing platform that can be used to track and discover biomarker patterns associated with stress and fatigue in personnel, which in turn will increase the safety and success of operations in harsh environments.

HIGH THROUGHPUT TESTING OF FACTORS AFFECTING BIOLOGICALLY CEMENTED MATERIALS

Authors:
Brandon M. Bradow, Matthew J Tuttle, Jessica Findsen, Rhett Martineau, Josh Mancini, Michael S. Carter, Karen Holley, Adriana Joson, Maneesh Gupta

Construction is a large producer of CO_2, and a lengthy process. Microbially-induced calcite precipitation (MICP) has been recognized as an alternative to traditional, CO_2 propagating cements for some time. By leveraging the work of bacteria, biocement can be implemented much faster, at room temperature, reversibility, and with lesser emission of CO_2. Recently, biocement has received attention for the capacity to make materials such as bio-bricks and ground improvement. The potential uses for biocement depend upon fast-paced testing and high-throughput testing devices. Low-carbon materials created using this technology may be useful for erecting impromptu structures, development of sites for humanitarian relief, or any other application requiring rapid, non-intrusive ground improvement. To investigate various methods feasibility for the creation of bio-bricks, a mold

is filled with the target soil type. A mesh retainer rests at the bottom of the column, which prevents soil from passing, but allows the percolation of necessary fluids. These fluids include bacteria, resuspended in water after cryopreservation – and cementation solution, which is a source of Ca^{2+} needed to precipitate calcite. For small scales, the pipetting of the cementation solution is outsourced to an Opentrons pipetting robot to increase throughput. Experimental manipulation includes the growth of the bacteria, soil conditions, factors like urease activity, and targeted depth. This method allows testing of high-impact questions at a low-stakes opportunity cost. Using this process, it is possible to alter formulations to target specific traits and features of biocement. We find that strength is largely proportional to the amount of Ca2+ administered to the system, in addition to such preconditions as urea access during growth, growth media type, and soil moisture. Counter-intuitively, urease activity has seemingly little effect. Some conditions thought to not be viable for cementation are now considered viable, with comparable strength values to the average 60-80 PSI (unconfined compressive strength, or UCS) achieved with conditions arbitrarily considered standard. UCS data upward of 300 PSI has also been collected while simultaneously reducing the "standard" material needs considerably.

LOW SIZE, WEIGHT, AND POWER (SWAP) SENSOR SELECTION FOR PILOT MASK CAPNOGRAPHY AND VALIDATION IN A SIMULATED AEROSPACE ENVIRONMENT

Authors:
Michael Brothers, Mark Aaron Hawkins, Paras Mainali, Zach Brooks, Steve Kim, Christin Duran

Pilot physiology monitoring is required to support mishap investigations, ensure pilot safety, and optimize pilot performance during flight operations. Capnography measures exhaled carbon dioxide (CO_2) to produce time series and end-tidal CO_2 data, which can be used for real-time in situ physiology assessment of breathing rates and to inform algorithms and digital models to characterize pilot medical status during flight. CO_2 sensors used in traditional capnography do not meet size, weight, and power (SWaP) requirements for pilot mask monitoring. Optimal sensor selection is required to deliver the capability for pilot mask physiology measurements during flight with minimal footprint and burden to the pilot. In this study, we conducted a literature review to develop a robust description of the principals of operation and prior art for CO_2 sensing. We also conducted a thorough market review to down-select candidate sensors for in-house performance evaluation. In addition to commercially available sensors, novel prototype sensors developed by academia, international partners, and industry were considered. Candidate sensors were evaluated in simulated breath at altitude in automated micro-chambers. Most low SWaP sensors suitable for pilot mask monitoring did not meet the response time requirements to be suitable for capnography. However, the data may still be useful to support algorithm development with the use of deconvolution techniques to compensate for slow response times. Future work is required to determine cross-sensitivities and calibration requirements for candidate sensors.

MULTI-OMICS FOR PERFORMANCE

Authors:

Only through a greater understanding of the Warfighter's biologic state and the molecular level of the underlying mechanisms can we enable, enhance, and sustain the Warfighter to ensure air, space, and cyberspace dominance. To address this need, the Airman Systems Directorate at the Air Force Research Laboratory (AFRL) has produced a multi-omic system by integrating a variety of "omic" datasets into prediction algorithms, simulation studies, and molecular discovery efforts. Omic information includes whole genome sequence data (their DNA), transcription profiles before and after exercise (RNA), proteomics (proteins made by that RNA and circulating in blood), and a variety of other related meta-data. By combining a multi-omics perspective, a robust and secure bioinformatics infrastructure, and the exercise physiology expertise of the Signature Tracking for Optimized Nutrition and Training (STRONG) lab, we have begun to identify novel associations and relevant biomarkers for specific, mission-relevant physiologic states. These biomarkers in turn will suggest specific molecular targets and pathways for intervention, as well as molecular candidates for sensor development. Ultimately, the system will be a secure, in-house, end-to-end, unified sample processing pipeline that supports large-scale, multi-dimensional analysis. This architecture will facilitate collaborations across the spectrum of research support needs within AFRL and with external collaborators. Further, the multi-disciplinary nature of our skilled workforce, from bench-to-report, will ensure ideal experimental design and high-fidelity analysis products. The AFRL multi-omic integration system will serve as a benchmark for DoD molecular research and open doors to impactful molecular discoveries.

CONDUCTIVE POLYMER SENSOR MADE IN 95 S FOR REAL-TIME DETECTION OF NEUROTRANSMITTERS IN HUMAN SERUM AND SALIVA

Authors:
Victoria E. Coyle[1,2], Michael C. Brothers[1,2], Zachary Brooks[1,2], Irina Drachuk[1,2], Sean Webb[1,2], Jorge L. Chávez[1] and Steve S. Kim[1]
Affiliations:
[1] 711th Human Performance Wing, Air Force Research Laboratory (AFRL), Wright-Patterson Air Force Base, Dayton, OH, USA
[2] UES, Inc., 4401 Dayton-Xenia Rd, Dayton, OH, USA

Email: steve.kim.13@us.af.mil

Electrochemical biosensors are rapidly becoming one of the key fields of science and innovation with the capability for people to self-monitor their health and well-being evermore important. Neurotransmitters are of particular interest as they can regulate mood, fatigue and cognitive function. When levels of neurotransmitters are irregular, certain medical issues can arise such as depression, addiction, anxiety disorders, Alzheimer's, epilepsy, and Parkinson's. Unfortunately, the extremely low concentration ranges for these analytes (low nM to low µM) in biofluids make it extremely difficult to create a sensor capable of detecting neurotransmitters. Due to this shortcoming our group has invented a new material which transitions off of the more commonly used conductive polymer PEDOT:PSS (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate) by omitting the PEDOT component and only depositing the PSS directly onto a glassy carbon (GC) sensing framework. When sensing serotonin the GC-PSS sensor had a sensitivity of 106.1 $\mu A.\mu M^{-1}.cm^{-2}$ which is 43 times greater

than the unmodified GC electrode and 6x larger than the GC-PEDOT:PSS sensor. Along with an improved sensitivity the GC-PSS sensor was now capable of detecting Serotonin concentrations as low was 10nM in PBS buffer and 50nM in pure human Serum. Further analysis of intermittent species determined that the GC-PSS sensor had no interaction with anything other than Uric acid and other neurotransmitters, leading to the discovery of multi-analysis detection of Serotonin, Tryptophan and dopamine within Serum concentration ranges. Further to this analysis of the GC-PSS sensor in saliva has shown great promise with Dopamine and Norepinephrine being detected. With the incorporation of a hydrogel matrix to encapsulate and filter the mucins the GC-PSS is able to detect neurotransmitters in a non-invasive biofluid as well making this novel sensor one of the most exciting steps forward in achieving a real-time neurotransmitter electrochemical sensor.
Key works: neurotransmitter, biosensor, electrochemical, saliva, Serum, conductive polymer

INJECTABLE HYDROGEL BIOSENSORS BASED ON FLUOROGENIC DNA PROBES FOR REAL-TIME STRESS DETECTION

Authors:
Irina Drachuk[1,2], Svetlana Harbaugh[1], Jorge L. Chávez[1]
Affiliations:
[1] 711th Human Performance Wing, Airman Systems Directorate, AFRL, 2510 5th Street, Wright-Patterson AFB, OH, United States
[2] UES, Inc.,An Eqlipse Company, 4401 Dayton-Xenia Rd., Dayton, OH, United States
POC Email: irina.drachuk.ctr@us.af.mil

Development of implantable sensors that can monitor analytes related to cognitive and physiological status has gained significant focus in recent years. We report on research related to the development of implantable Forced Intercalation (FIT)-Aptamers for autonomous monitoring of biomarkers associated with physical performance. The mechanism of light-up aptamer is based on intramolecular conformational change during forced-intercalation of viscosity-sensitive dye between oligonucleotide base pairs during aptamer-target binding event leading to its fluorescence turn on. For the FIT-Aptamer to be functional in the hydrogel, the matrix should not restrict a structure switching mechanism and allow the formation of an aptamer binding pocket. Several biocompatible hydrogel formulations based on synthetic and natural polymers were selected to investigate the effect of the polymer matrix on the function of a structure switching light-up aptamer. The synthetic polymer network, poly(ethylene imine) (PEI) crosslinked with poly(ethylene glycol) (PEG) and natural glucosamine glycan network, hyaluronic acid (HA) polymers were characterized to yield hydrogel matrix. We optimized the hydrogel formulations to produce optically transparent network with optimal mesh size, structure, mechanical properties, and gelation-time transitions for optimal analyte sensing. The optimization of a sensor function was performed using a model fluorescence turn-on DNA aptamer responsive to a crystal violet dye. Following the optimization with model aptamer, we confirmed an activation of FIT-Aptamer for steroid dehydroepiandrosterone sulphate (DHEA-s). We demonstrate that hydrogels with optimal mesh size, structure, hydrophilicity, and mechanical properties can preserve the aptamer conformation during analyte binding which leads to generation of a fluorescent signal within 30-40 min.
Key words: DNA, force-intercalation aptamer, fluorogenic aptamer, hydrogel, stress sensing.

A POINT-OF-USE SALIVARY ALLOSTATIC LOAD SENSOR

Authors:

Allostatic load is the process of adaption from acute stress and can be measured via presence and concentration of various biomarkers, such as cortisol and aldosterone. By tracking the allostatic load of a war fighter, their stress level can be monitored, and restorative action can be taken when levels are too high, which is linked to high fatigue and poor decision making. Biological samples can be conveniently taken from the war fighter via spitting into a tube, which reduces the stress often resulting from invasive methods. Here, cortisol or aldosterone was detected via a DNA aptamer biorecognition element (BRE), which was optimized for detection in saliva. A methylene blue (MB) redox marker is attached to the aptamer and acts as the biosensor transduction mechanism, and upon binding to target, a structural change brings the MB closer to the sensing surface of the electrode. This structural change results in a higher peak response that is proportional to the concentration of the target in saliva. The saliva sample was processed for electrochemical measurement with a 30,000 Dalton molecular weight centrifugation filter to remove possible interferents or biofouling components and with a polyethyleneimine polyethylene glycol diglycidyl ether hydrogel coating on the electrode surface. These processes doubled the sensitivity of the biosensor, affording the detection of cortisol in the filtered spit in the range of 1-100 nM. Overall, the use of electrochemical sensing techniques with aptamer BREs provides a method for biochemical analysis to quick turnaround time, portable device setup, and minimal sample prep.

MICROBIOME CHANGES CORRELATE WITH HUMAN HEALTH AND PERFORMANCE MEASURES DURING A SEVEN-MONTH SUBMARINE DEPLOYMENT

Authors:
Dr. Zachary Liechty[1], SSgt. Arianna Baldwin[1], Dr. Blake Stamps[2], Ms. Rhonda Pitsch[1], LT Mahamat Babagana[3], LCDR Joseph Decicco[3], LCDR Neal McNeal[3], Dr. Michael Goodson[1], Surg CDR Joanna Halford[3]
Affiliations:
[1]711th Human Performance Wing, AFRL
[2]Materials and Manufacturing Directorate, AFRL
[3]Naval Submarine Medical Research Unit

If you woke up this morning feeling less than human, you'd be right! We are all mostly microbe: you have slightly more microbial cells in and on your body than human cells. There are over 2 lbs of microbes residing in your gut alone! We all carry our own personalized mix of different kinds that we have acquired over our lifetimes, and whose diversity was built up from birth by exposure to family members, food, pets, and our environment. These microbes are essential for survival! They do all sorts of things for us: they digest parts of our diet that we cannot; they make essential vitamins that we need; and they stop invading pathogens. Every day, your human self and your microbial self use molecules as words to have a conversation with each other. Humans evolved in a microbial world so it shouldn't be surprising that we speak the same language. The conversations are sometimes slow, such as when communication occurs using molecules in the blood stream, but they can also be fast, such as when the words initiate firing of the nerves between the gut and the brain. In fact, the gut is the second most innervated organ in our body after our brain, and has even been termed 'our second brain', so not a bad place for our 2 lb of microbes to send and receive signals from. Your 'gut feelings' may actually be messages from your gut microbes! If these conversations turn into expletive-laden yelling matches, the result is not only a 'dodgy tummy' whilst on holiday, but it can affect our mood,

our cognition, our tolerance to pain, and even ability to focus. The best way to keep a healthy conversation going with your microbial self is to do the things that your mom has always told you to do: Eat more vegetables; Get more sleep; Do more exercise (yeah, who knew that moms were always right?!). The problem for our deployed warfighters is that often they do not have the luxury of achieving all or any of these. Consequently, their internal dialogue with their microbes can be a little off, resulting in health issues, lowered mood, reduced cognition, reduced pain tolerance, and reduced focus; all things that they need to rely on to complete their mission.One of the hardest things about studying microbiomes is that humans are terrible research subjects: they rarely stick to the same diet; they don't like to be in the same place every day; and they all have different activity levels. It's a nightmare! Consequently, we looked for a deployed environment where these factors weren't so much of an issue. The answer? Submarines. A submarine is a well-defined environment: submariners are not likely to experience wildly different diets, stresses, or environments than their crewmates. And, since AFRL is one lab supporting two services, there is no better proxy to manned spaceflight on Earth than a submarine: limited diet, altered circadian rhythm, poor sleep, stress, lack of exercise, and lack of vitamin D. All these factors are known to affect our microbiome. Thirty U.S. Navy submariners, serving on a fast attack submarine (SSN), provided fecal samples, blood samples, and skin swabs; completed questionnaires relating to their diet (Block Food Frequency Questionnaire) and mood (abbreviated Profile of Mood States); and completed serial cognitive assessments (Automated Neuropsychological Assessment Metrics) before, during, and after a seven-month deployment. Objective sleep data were also collected during the deployment, using actigraphy watches (Motionlogger Micro Watch). Environmental sampling of various surfaces aboard the submarine was also conducted before and during the deployment, to coincide with the skin swabs from the subjects, to explore the effects of the environmental microbiome on the human microbiome. Analysis of the fecal, skin, and environmental samples included DNA-based sequencing to identify the microbes present; and for the fecal samples, we also used digital PCR techniques, to determine absolute abundances of those microbes. High throughput metagenomic sequencing of the fecal samples was done to assess the functional capacity of the microbes present, and untargeted metabolomics analysis was used to assess the functional activity of the microbes. The blood samples were analyzed to identify metabolites and proteins of interest, focusing on those with established links to stress, neurocognitive function, immune function, and gut barrier function. The dietary and mood questionnaires, actigraphy data, and cognitive assessments were analyzed to identify any changes over the course of the deployment, and any correlations between those changes and changes in the microbiome. Preliminary results indicate that, while the microbiome is highly individualized, deployment did significantly impact its composition. Furthermore, the microbiome of Service members significantly correlated with various aspects of mood, eating habits, and cognition, suggesting that the microbiome may influence health and performance. You may be wondering how is this helping our warfighters? Unlike the information contained in our own cells, it is way easier to change who is in our microbiome and what our microbes do. Armed with the knowledge gained by people pooing for science, we can design targeted dietary interventions to promote healthy and resilient microbiomes.

SYNTHETIC BIOLOGY SOLUTIONS FOR PERFORMANCE SENSING AND AUGMENTATION

Authors:

Svetlana V. Harbaugh[1], Kathryn R. Beabout[2], Irina V. Drachuk[2], Natasha L. Kennel[2], Jorge L. Chávez Benavides[1]

Affiliations:

[1]711th Human Performance Wing, Wright-Patterson Air Force Base, OH, USA

[2]UES, Inc., Dayton, OH, USA

POC Email: svetlana.harbaugh.4@us.af.mil

The USAF and USSF recognize the critical role that Airman and Guardian stress, fatigue, and cognitive overload play in mission success. Deployed personnel must perform long missions in challenging environments with limited resources, which critically affects their performance. Biomarker levels associated with fatigue, stress, and cognition can be used to assess personnel conditions and create interventions that allow the Airmen and Guardians to perform at high levels over multi-day missions with minimal logistics support.
Biology offers a rich source of sensing modalities and components that can be used to build powerful real-time sense-and-respond platforms that offer significant advantages over traditional sensors. Our team is applying synthetic biology for the development of biosensors that allow for the detection of health and performance biomarkers in biofluids. By employing several molecular sensing approaches, we developed cell-free biosensors that detect deoxycholic acid, dopamine, progesterone, γ-aminobutyric acid. These cell-free sensors provide modular and fieldable sensing platforms that are ideal for point-of-use (POU) detection; they are stable once freeze-dried and can be transported to austere locations without a cold chain and rehydrated with appropriate biofluid onsite. To measure and augment performance in real time, we build a wearable sensor based on the microneedle patch made of biocompatible polymer. The bio-patch contains engineered bacteria programmed to produce mitigation compounds in response to biomarker detection in interstitial fluid. The engineered bacteria are capable to detect a human stress biomarker, cortisol, and release a cognition-specific neurotransmitter, serotonin, to counterbalance the issue.
Key words: *biosensor, performance biomarkers, microneedle bio-patch.*

TOPOLOGICAL DATA ANALYSIS FOR IDENTIFICATION OF MICRORNAS AS BIOMARKERS FOR HUMAN PERFORMANCE

Authors:
Christopher Dean Ph.D., Rajesh R. Naik Ph.D. & Ryan M. Kramer Ph.D.

Micro-RNAs (miRNA) are non-coding RNAs that play a crucial role in regulating gene expression and have been linked to various physiological processes, including physical performance. However, miRNA expression profiles are highly nonlinear and high-dimensional, making it difficult to analyze them using conventional statistical methods. This seedling aims to explore the potential use of topological data analysis (TDA) as a robust approach to analyzing microRNAs (miRNAs) associated with human performance. TDA can capture the complex topological structure of miRNA expression data, identify important topological features, and integrate related features and metadata from multiple sources, thereby improving the reliability and robustness of the analysis. We propose the development of a deep topological machine learning algorithm based on TDA, combined with hierarchical decomposition, to uncover differences and relationships in miRNA expression patterns in subjects with traditional and high-intensity training. We hypothesize that using TDA, we will be able to provide richer insights into the functional role and signaling pathways of miRNAs identified for physical performance.

HYPER ENABLED INSTRUCTOR

Authors:
Colleen Thamm, Tyler Van Hook, Matt Ackerman

Confronting unique challenges in today's dynamic operational environment, the Air Force is prioritizing data analytics to enhance training and readiness. Amid increasing recruitment and attrition issues, the shift towards advanced training technologies is crucial. This approach aligns with the Air Force's mission, ensuring preparedness for future challenges and upholding its legacy of excellence.

A key innovation in this endeavor is the Hyper Enabled Instructor (HEI), integrated within the Microsoft Suite. HEI revolutionizes training by providing comprehensive insights into the lifecycle of Air Force personnel, aiding in decision-making and enhancing overall effectiveness.

HEI's predictive analytics model is instrumental, allowing for early identification of learners needing additional support, thereby ensuring mission success. Additionally, continual improvements in training are achieved through data analysis, leading to more effective learning experiences and economically responsible investments.

This strategic adoption of data-driven methods signifies a major advancement in Air Force training, equipping airmen for today's complex scenarios and future operational demands.

SENSING CORTISOL AT THE TIP OF MICRONEEDLES IN HUMAN SKIN

Authors:

Trevor B. Tilly[1], Victoria E. Coyle[1], Irina Drachuk[1], Steve S. Kim[1], Lawrence Drummy[2], Jorge L. Chávez[1]

Affiliations:

[1] 711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio 45433, USA

[2] Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio 45433, USA

POC Email: trevor.tilly.1@us.af.mil

The development of wearable sensors utilizing engineered microneedles (MNs) for minimally invasive interstitial fluid (ISF) extraction and the simultaneous identification and quantification of relevant stress and fatigue biomarkers by electrochemical aptamer-based (E-AB) sensors is critical in enabling enhanced human performance of Airmen and Guardians. A proof-of-concept wearable MN and E-AB device was developed for the detection of cortisol due to its significance as a key stress biomarker. On-body cortisol measurements will be achieved by introducing the specific DNA aptamer biorecognition element into the dermis-contained ISF via the MNs. This procedure affords continuous and real-time sensing. Here, the E-AB sensor was optimized for rapid measurement of cortisol within its physiological range of 1 to 500 nM. Squarewave voltammetry was employed as the electrochemical measurement technique for rapid (<10 seconds) measurement of cortisol in ISF using an aptamer bound methylene blue redox reporter. The electrodeposition of gold nanostructures on the electrodes enhanced the sensor signal by over five-fold in comparison to smooth flat gold electrodes. Sensor drift was reduced by over 10-fold by adding a hydrophilic polymer hydrogel coating over the electrode bound aptamers. Further development of the MNs and integration of the E-AB sensor into an on-body platform will result in a high utility wearable device that is efficient in the long-term monitoring of human performance to achieve the highest level of warfighting readiness.

Key words: *Biosensor*

A PLATFORM FOR RAPID DESIGN AND DISCOVERY OF BRES FOR EMERGING THREATS

Authors:
Sean Webb[1,2], Monica Wolfe[1,2], Alyssa Cramer[1,2], Yaroslav Chushak[1,3], Peter Mirau[4], Jorge Chavez[1]
Affiliations:
1)711th Human Performance Wing, AFRL;
2) UES, Inc. Dayton, OH;
3) Henry M. Jackson Foundation, Dayton, OH;
4) Materials and Manufacturing Directorate, AFRL

In a world of global pandemics and increased biological weaponization, the need for rapid and accurate diagnostics is more pertinent than ever. One component common to all sensing platforms is the bio-recognition element (BRE), which sits at the interface between sensor and the sample matrix and is responsible for detecting the presence or absence of the target molecule. While this role has traditionally been filled by antibodies, aptamers are a class of BRE gaining favor due to their thermal stability, low immunogenicity, and ease of synthesis. Aptamers are unique sequences of oligonucleotides (DNA/RNA) or amino acids (peptides) with a specific three-dimensional structure and potential for target binding. To improve the traditional aptamer selection process, we have established a platform for high-throughput selection designed to rapidly respond to emerging threats. Here, we use *in silico* structural and computational modeling to generate large libraries of sequences with predicted binding to a target of interest. Screened in parallel for binding using microarrays holding up to 1 million sequences, promising sequences are then down selected for comprehensive binding characterization using techniques such as bio-layer interferometry (BLI), isothermal titration calorimetry (ITC), and/or ELISA. Here we show results for a range of targets including viral proteins, biotoxins, and small molecule biomarkers with target-binding affinity at levels relevant for operational sensing and have been transitioned into a variety of sensing platforms. As new biological threats or biomarkers are identified, we believe this platform will enable the rapid discovery of BREs required for development of sensors and diagnostics.

Acknowledgements
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Symposium: Human Performance (Demo Presenters)

ADVANCED HYDRATION SYSTEMS & METHODS FOR OPTIMAL HUMAN PERFORMANCE - FROM NASCAR TO THE BATTLEFIELD

Authors:
David P. Ferguson, PhD & Kevin R. Mantovani, Col (Ret), USAF

Astounding levels of Human Performance are achievable through the precise timing and micro dose consumption of just water.
FluidLogic is a late-stage biotech startup that builds the worlds-only "Active" Hydration systems, used by NASCAR, Indy Car and most other professional racing teams to predict when and how much water a driver must drink (through an advanced predictive analytics algorithm) and through an automated fluid delivery system that provides users with hands-free and thought free drinking that enables them

to achieve and maintain optimal cognitive and physical performance during grueling racing events up to 26 hours (Baja 1000 race) long. Dr. Ferguson will present the results of his recently published (Oct 23 - Journal of Sports Sciences) Clinical Study comparing the effects of micro-dosed water consumption using FluidLogic during a simulated race versus traditional drinking. The results of this peer-reviewed study conducted at the Michigan State University Performance Motorsport Laboratory were ground-breaking and indicate vastly improved cognitive and physical performance, improved endurance and reduced recovery time (post-event) using FluidLogic. Col (ret) Kevin Mantovani will then demonstrate and discuss FluidLogic's 2022 R&D (AFWERX SBIR) prototyping effort with USAF Ground Combat personnel - a Tactical Advanced Hydration Ecosystem that was recently called "The Top Technology" at the 2023 DTRA Chemical Biological Operations Analysis event. They will conclude the presentation with a brief discussion of R&D opportunities to collaborate on with AFRL that address top USAF and DoD capability gaps (such as Maximum Endurance Operations) and can result in rapidly fielded systems for personnel in Aircraft, CBRN, Arctic environments, etc.

DEPLOYABLE BIOHEATER FOR TEMPERATURE REGULATION IN EXTREME COLD

Authors:
Veronica Williams[1,2], Kathryn Beabout[1,2], and Jorge L. Chávez[1]
Affiliations:
[1] US Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, USA
[2] UES, Inc., 4401 Dayton-Xenia Rd, Dayton, OH, USA

Operations in the Arctic are expected to increase due to recent geopolitical events. This extreme environment leaves personnel vulnerable to cold-related injuries, such as hypothermia, and disrupts equipment that was not designed to operate in frigid temperatures. Therefore, an autonomous lightweight and portable heat source could greatly improve the safety and efficiency of operations in extremely cold environments. To generate such a heat source, we have engineered the alternative oxidase respiratory pathway, which is a main driver of heat production in many thermogenic plants, in an industrially used strain of yeast, Pichia pastoris. We have shown that our engineered P. pastoris can generate about 10 times as much heat as the parent strain, highlighting the utility of the alternative oxidase pathway for increased heat production. To further enhance heat generation, we have shown that optimizing the growth conditions of our engineered yeast improved heat output. By introducing an electron transport chain inhibitor to the yeast, which blocks the normal respiratory pathway and employs only the alternative oxidase respiratory pathway, we have shown that optimizing the energy pathways also improved heat output. Our engineered yeast can be incorporated into hydrogels, fabrics, and other materials to generate living clothing and gear that can maintain personnel and their equipment at ideal temperatures in extremely cold environments. The development of the technology could greatly enhance the safety and efficiency of operations in extremely cold environments.

Symposium: Materials (Oral Presenters)

BIOPRINCIPIC LESSONS FROM INVERTEBRATES

Authors:
Laura Bagge, Nathan Lord, Nick Rummelt, Martin Wehling

The astonishing diversity of form and function in invertebrate sensory systems, particularly insect visual systems, can inspire improved designs of man-made sensors. Studying the underlying mechanisms for how animals may be able to perceive otherwise private channels may result in significant technological advancements for bioinspired target detection in engineered systems. Insects are one of the most diverse and successful organisms on the planet, and within them, the beetles occupy nearly every role and location in the environment. Beetles are superb proxies for bioprincipic design, as their armored exteriors, ability to fly, and expanded sensory capabilities align extremely well with the armaments needed to equip the warfighter for success. Combine this with the fact that insects are in a near-constant state of both defensive and offensive engagement and conflict for survival, it becomes imperative we learn from some of nature's most long-lived successes. AFRL/RWTCA's Nature-Inspired and Algorithms Team has recently worked with OSR-funded collaborators in Costa Rica and with AFRL's "University-Day" awardees in the US on topics related to both unique optical signatures and the toughness of the cuticle of beetles.

BIOLOGICALLY-INSPIRED MULTIFUNCTIONAL ARCHITECTED MATERIALS – AND THEIR TRANSLATION

Authors:
David Kisailus[1]
Affiliation:
[1] *Department of Materials Science and Engineering, University of California, Irvine, USA*

[*]*Corresponding Author's E-mail: david.k@uci.edu*

Organisms have derived specific sets of traits in response to common selection pressures that serve as guideposts for optimal biological designs. A prime example is the evolution of toughened structures in disparate lineages within plants, invertebrates, and vertebrates[1]. Extremely tough structures can function much like armor, battering rams, or reinforcements that enhance the ability of organisms to win competitions, find mates, acquire food, escape predation, and withstand high winds or turbulent flow. Some of these natural systems have developed well-orchestrated strategies, exemplified in the biological tissues of numerous animal and plant species, to synthesize and construct materials from a limited selection of available starting materials. The resulting structures display multiscale architectures with incredible fidelity and often exhibit properties that are similar, and frequently superior to, mechanical properties exhibited by many engineering materials[1,2]. In specific instances, comparative analyses of multiscale structures have pinpointed which design principles have arisen convergently; when more than one evolutionary path arrives at the same solution, we have a good indication that it is the best solution. This is required for survival under extreme conditions. We describe a few of these systems that show convergent design and describe how controlled syntheses and hierarchical assembly using organic scaffolds lead to these integrated macroscale structures[3-8]. We describe their function and translation to biomimetic and bioinspired materials used for engineering applications (Figure 1) [9-11].

Figure 1. Translation of Biological Designs to Engineering Applications

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PROTEIN-MEDIATED POLYPHOSPHATE SYNTHESIS AND TEMPLATING OF NANOSTRUCTURED BIOCOMPOSITES

Authors:
Peter A. Mirau, Patrick Dennis, Sanaz Farajollahi, and Joe Slocik

Functional nanostructures in nature are often constructed from proteins and inorganic materials templated to obtain composites with superior mechanical or optical properties. We are creating composites by non-enzymatically cross linking energy-rich polyphosphate (polyP) with low complexity PolyAcidic, Serine and Lysine (PASK) domains in selected proteins. We have expressed portable PASK domains with linkers to self-assemble into nanostructures, and demonstrated their reactivity with polyP by gel electrophoresis. The PASK domains contain a number of possibly reactive lysine residues and we have been using analytical methods (including NMR, Biolayer Interference, and Quartz Crystal Microbalance) to understand structure-property relationships and to characterize the reaction products. We are evaluating the thermal properties of the biocomposites as possible ablative materials for space craft repair. By understanding the reaction mechanism and reaction products we plan to optimize the properties of composites for Air Force applications.

IRIDESCENT BIOFILMS OF BACTERIA: VERSATILE PLATFORMS FOR FUNCTIONAL MATERIALS

Authors:
Claretta J. Sullivan[1], Kennedy Brown[1], Chia Hung[1], Joseph Kuo-Hsiang Tang[1], Mark DeSimone[1,2], Vincent Chen[1], Pam Lloyd[1], Maneesh Gupta[1], Abby Juhl[1], Wendy Crookes-Goodson[1], Milana Vasudev[1,2], Patrick Dennis[1], Nancy Kelley-Loughnane[1]
Affiliations:
[1] Materials and Manufacturing Directorate, Air Force Research Laboratory, WPAFB, OH 45433 U.S.A.
[2] Department of Bioengineering, University of Massachusetts Dartmouth, Dartmouth, MA 02747 U.S.A.

Nature offers many examples of materials which exhibit exceptional properties due to hierarchical assembly of their constituents. In well-studied multi-cellular systems, such as the morpho butterfly, a visible indication of the requisite scale and ordering is given by the display of structural color. Detailed investigations of nature's designs have yielded mechanistic insights and led to the

development of biomimetic materials at laboratory scales. However, the manufacturing of hierarchical assemblies at industrial scales remains difficult. Solutions may be available through biomanufacturing which aims to leverage the autonomy of biological systems to produce materials at lower cost and with fewer carbon emissions. Structural coloration is an emergent property linked to the *Flavobacteriacea* family of bacteria including genera *Cytophaga*, *Flavobacterium* and *Cellulophaga.* Under suitable conditions, these bacteria self-assemble into ordered 3-dimensional biofilms, displaying structural color with an angle-dependent peak intensity. This so called iridescence derives from the interactions of light with the hierarchically assembled cells. Color generation is also dependent on the gliding motility of the bacteria though the precise mechanisms involved in forming crystalline biofilms are currently under study. We have identified laboratory conditions that allow biofilms of *Cellulophaga lytica* 7489 to reliably generate a range of colors. Importantly, *C. lytica* biofilms form in ambient conditions and across multiple length scales. We predict that this facile growth can be used to overcome challenges hindering the mass production of ordered materials. In the current study, approaches for controlling biofilm optical and spatial properties are reported. Since the bacterium is the structural unit, dynamic iridescence is achieved through changes in cell morphology prompted by augmented growth conditions. This study is an important report of making materials using *C. lytica* as we demonstrate their utility as self-printing photonic inks.

ADVANCES IN PROTEIN-BASED BIOMATERIALS FOR DOD APPLICATION

Authors:
Sanaz Farajollahi

Nature offers inspiration for discovery and development of complex advanced materials to address the grand challenges in sectors such as military, energy and environment. Dynamic structural tunability of many natural proteins such as silk, collagen, keratin, and etc. provides advanced mechanical, chemical, electrical, electromagnetic, and optical properties that are superior to synthetic polymers. Thus, protein derived biomaterial creates an attractive alternative to synthetic polymeric material, due to their programmable structural and functional properties. By harnessing the power of synthetic biology tools and bioinspired fabrication of structural proteins, novel materials with smart functionality can be created to serve diverse application. Here, we highlight some of the advances as well as the challenges in development of bioinspired materials for DOD applications.

Symposium: Materials (Poster Presenters)

ELASTIN-LIKE POLYPEPTIDE (ELP) BIOELASTOMER-ASSISTED SIMPLIFIED EXTRACTION AND PURIFICATION OF RARE EARTH ELEMENTS

Authors:
Loryn L. Bowen[1], Adam M. Reed[1], Oscar N. Ruiz[2], Chia-Suei Hung[2], Andrea Hoffmann[1] and Kara L. Martin[3]
Affiliations:
1. University of Dayton Research Institute, Dayton, OH
2. RXEB, Biomaterials Branch, Photonic, Electronic & Soft Materials Division, Materials & Manufacturing Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH

3. RQTF, Fuels & Energy, Turbine Engine Division, Aerospace Systems Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH

POC Email: loryn.bowen.1.ctr@us.af.mil

Low-cost storage technologies and more efficient engines are critical for energy generation from renewable sources. Rare earth elements (REE) have been identified as resources needed for battery energy storage units, consumer electronics and electric vehicles. The harvest and recycling of rare elements has become of increasing demand for use in military and aerospace applications such as energy-saving lamps, lasers, radar, computer drives, electric motors, and as catalytic converters in industrial applications. This study tests a novel methodology that uses the recombinant Elastin-like Polypeptides (ELP) fused to metal affinity peptides such as the lanthanide binding protein LanM for elastomere-assisted purification of lanthanide REE such as Nd, La, Tb, Dy, Yb, Eu.
Key Words: elastin-like polypeptide, bioelastomer, LanM, rare earth elements.

OPTIMIZING BIOCEMENTATION THROUGH CHARACTERIZATION OF UREOLYTIC ACTIVITY

Authors:
Kristen Bruce[12], Michael S. Carter[12], Mathew J Tuttle[12], Maneesh Gupta[1], Rhett Martineau[1]
Affiliations:
1Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH 45433
2Biological and Nanoscale Technologies Division, UES Inc., Dayton, OH 45432

Concrete is the second most consumed material on the planet and is estimated to produce 8% of global carbon emissions. Biocement is a type of building material that has the potential to be a greener option than traditional concrete, reducing emissions of this widely used resource. Biocementation is accomplished by Microbial Induced Calcite Precipitation (MICP). This is a process that uses ureolytic microbes and a chemical feedstock that contains urea and calcium to produce calcium carbonate. The calcium carbonate binds aggregates to produce biocement. Sporosarcina pasteurii is the most widely used organism for this process due to its high urease activity, alkaline tolerance, and ability to survive in the presence of the toxic ureolytic waste product, ammonia. Though S. pasteurii is widely used for biocementation there are still many questions about what biochemical and genetic pathways it employs to achieve this. This study focuses on the urease enzyme and urea's role in the organism. Our team is actively sequencing the S. pasteurii genome, and we are comparing global gene expression and urease activity in various growth conditions. Some preliminary conclusions show that the urease enzyme is not genetically regulated in different growth conditions. It also appears that the urease gene cluster has evolved independently of other organisms that perform urea hydrolysis. We are developing a genetic toolkit to further investigate the genome of S. pasteurii. When these strategies are developed, we will employ the toolkit to learn more about what role urea plays in this organism's biochemistry and how urea is transported into the cell.

MONITORING SPOROSARCINA PASTEURII PERCOLATION THROUGH SAND COLUMNS VIA UREC QPCR.

Authors:
Eleanor K. Chase[1,2], Matthew J. Tuttle[1,2], Kristen A. Bruce[1,2], Jessica L. Findsen[1,2], Brandon M. Bradow[1,2], Michael S. Carter[1,2], Maneesh K. Gupta[1], Rhett L. Martineau[1]
Affiliations:
1Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH 45433
2Biological and Nanoscale Technologies Division, UES Inc., Dayton, OH 45432

Globally, cement production constitutes an immense environmental footprint, contributing an estimated 8% of total greenhouse gas emissions annually. A factor of these high emissions is cement's short lifespan as cracks in traditional cement cannot be sealed so structures require frequent maintenance and replacement. Biocement has potential to mitigate these issues by offering a low carbon alternative with self-healing capabilities. Biocement is a cement-like material consisting of an aggregate fused together through Microbially Induced Calcium Carbonate Precipitation (MICP). We are developing a method to utilize existing sandy soils as an aggregate and inoculating them with *Sporosarcina pasteurii* to induce in-situ cementation*. S. pasteurii* converts urea feedstocks to carbonate ions via a urease enzyme. The carbonate reacts with calcium ions, forming a network of $CaCO_3$ crystals that harden the soil into biocement. This creates rapid soil stabilization without heavy machinery or mechanical disturbance. A major challenge is achieving uniform percolation of bacteria through the soil, which limits the strength of the resulting cement. Monitoring the distribution of *S. pasteurii* in the soil therefore helps indicate biocementation potential and provides a testable metric for further exploration. In lab studies, we monitor bacterial percolation with 24-inch sand column studies and have modified variables such as moisture content, cell density, salinity, temperature, and post-inoculation flushes. We then extract DNA from the soil and measure relative abundances of the *ureC* gene through the full column depth via qPCR. Additional variables of interest include soil compaction, texture, and organic matter content which may provide insight into more diverse soil types for biocementation. Preliminary results suggest flushing the columns after inoculation is an effective method for achieving a deeper and more uniform biomass distribution and may produce stronger cement.

PROCESS OPTIMIZATION OF *SPOROSARCINA PASTEURII* BIOMASS PRODUCTION FOR BIOCEMENTATION

Authors:
Jessica Findsen[1,2], Adriana Joson[1,2], Michael Carter[1,2], Rhett Martineau[1], Maneesh Gupta[1]
Affiliations:
[1] Air Force Research Lab, Materials Directorate, Wright-Patterson Air Force Base, OH
[2] UES, Inc., Biological & Nanoscale Technologies Division, Dayton, OH

Traditional concrete production contributes up to 8% of global CO_2 emissions. Biocement is an environmentally friendly cement alternative produced by bacteria and is currently under investigation for industrial use. Biocementation has traditionally been performed via microbially induced $CaCO_3$ precipitation (MICP), which utilizes enzymatic reactions, biological superstructures, and feedstock material to produce $CaCO_3$ within aggregate materials. The current method of biocementation requires growth and immediate application of the bacteria to the aggregate at the site of biocementation. Optimization of biocement production involves large-scale growth of the bacteria and freeze-drying of produced biomass that can be transported to the field. This study

describes production of *Sporosarcina pasteurii*, the workhorse organism of biocementation, and growth trends at a 10-liter bench-scale. Process optimization of *S. pasteurii* production gives insight to maximization of industrial biomass production. Current efforts have reduced the production of a toxic byproduct, reduced the cost of biomass production by switching to an alternative growth medium, and determined an optimized method of biomass freeze-drying. Future studies can utilize growth trends to increase total biomass production in a time- and resource- conducive manner.

POLYMERIZED HUMAN HEMOGLOBIN-BASED OXYGEN CARRIER PRESERVES LUNG ALLOGRAFT FUNCTION DURING NORMOTHERMIC *EX VIVO* LUNG PERFUSION

Authors:
Clayton Cuddington[#1], Alisyn Greenfield[#1], Yong Gyu Lee[2,3], Jung Lye Kim[2,3], Derek Lamb[4], Paul W. Buehler[4], Sylvester M. Black[2,3], Andre F. Palmer[1], Bryan Whitson[2,3,5]
Affiliations:
#Equal contribution, co-first authors
1 William G. Lowrie Department of Chemical and Biomolecular Engineering, College of Engineering, The Ohio State University
2 Department of Surgery, The Ohio State University Wexner Medical Center
3 The Collaboration for Organ Perfusion, Preservation, Engineering and Regeneration (COPPER) Laboratory
4 Departments of Pathology and Pediatrics, Center for Blood Oxygen Transport Hemostasis, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, MD, 21201, USA
5 The Davis Heart and Lung Research Institute at The Ohio State University Wexner Medical, College of Medicine

There is a significant disparity between the number of patients on the national transplant list and the number of organs available for life-saving transplantations. This is due to several factors including geography, number of donors, and type of donor death. To improve upon this discrepancy, there must be an increase in the number of organs donated or an improvement of the organs initially deemed not viable for transplantation. One method that has been developed to improve lung viability is normothermic *ex vivo* lung perfusion (EVLP). This system integrates a donated lung into an extracorporeal circuit that cycles a perfusate solution at physiological temperature to maintain lung metabolic functions. EVLP aids in limiting ischemia reperfusion injury that is commonly associated with the traditional static cold storage technique for organ preservation. By circulating a perfusate solution through the lungs, nutrients can be provided to the organ to maintain native metabolism while also clearing metabolic waste products to sustain organ function *ex vivo* for an extended period. In addition to maintaining organ metabolism, EVLP has demonstrated a capacity to monitor and rehabilitate organs that have initially been deemed non-viable for transplantation upon initial inspection; overall, improving the number of organs available for transplantation.
Previous literature has used either a colloid solution alone or a colloid solution supplemented with red blood cells (RBCs) to aid with oxygen delivery. The addition of RBCs in the perfusate for EVLP has demonstrated to be beneficial in providing oxygen to the tissues to maintain metabolism thus becoming a clinical standard. The limitation of using RBCs in the perfusate are two-fold: 1) RBCs are a scarce resource; and 2) are prone to lysis on the perfusion circuit, releasing cell-free hemoglobin (Hb) that extravasates into the tissue space where it scavenges nitric oxide and leads to increased pulmonary arterial pressure and pulmonary vascular resistance. This work proposes an alternative

oxygen carrier, polymerized human hemoglobin (PolyhHb), for use as a perfusate supplement in EVLP. PolyhHb is a Hb-based oxygen carrier that is synthesized via a crosslinking reaction to make large protein aggregates. PolyhHb is purified via tangential flow filtration to be bracketed between 500 kDa and 0.2 µm in size, thus limiting extravasation of the material into the tissue space. PolyhHb has all the oxygen carrying capabilities of RBCs without the risk of mechanical lysis in the circuit and can be manufactured at scale, making it readily available.
This work compares a next-generation PolyhHb-based perfusate against an RBC-based and an asanguinous control perfusate in a rat EVLP model. Throughout the perfusion, the pulmonary arterial pressure and pulmonary vascular resistance were significantly higher in lungs perfused with RBCs, results consistent with hemolysis. Lungs perfused with PolyhHb-based perfusate displayed improved tissue oxygenation compared to those perfused with RBCs. Post-EVLP analysis revealed that the PolyhHb-based perfusate elicited less cellular damage, tissue extravasation, iron tissue deposition, and edema formation than either RBCs or control perfusates. These results are promising for future application of a next-generation PolyhHb to maintain lung function during EVLP.

USING FOUR-HELIX BUNDLES PROTEINS AS A NOVEL PROTEIN ENGINEERING PLATFORM FOR SELECTIVELY ISOLATING RARE EARTH ELEMENTS

Authors:
Joshua Mancinia,b, Maya Raoa,b, Emily Koernera,b, Martha Cartera, Nancy Kelley-Loughnanea, Selemon Bekelea,b, Hao-Bo Guoa,b, Rajiv Berrya, Courtney Henthornc Justin C. Biffingerc , Chia-Suei Hunga

Lanmodulin (LanM) is a highly selective lanthanide binding protein from Methylorubrum extorquens AM1. This binding occurs using 3 EF-hand loop domains that are tailored specifically to the size and coordination environment of lanthanide metal ions. The acidic residues, glutamate and aspartate, are the residues found in these domains that comprise most of the coordinating residues specific for lanthanide metals. We tested the potential for loop 1 of LanM to be able to function independently of the rest of the LanM structure. Four-helix bundle proteins were chosen as a potential framework for grafting LanM loop1 into. Four-helix bundle proteins are connected by 3 flexible loops which have been shown to be amenable to extensive modification such as length and charge. We chose the four-helix bundle with pdb id: 8D9O as the chassis for LanM loop grafting. Intrinsic tryptophan quenching upon lanthanide binding demonstrated successful Dysporsium, Terbium, Europium, Neodinium, and Lanthanum binding with dissociation constants in the low nanomolar range and a preference for larger ionic radii REEs. This work demonstrates that the lanthanide metal binging loop region of LanM is modular. As long as the loop is held semi rigidly by 2 alpha helices in parallel orientation this is enough to drive lanthanide metal binding. The four-helix bundle has two other loop regions where 2 additional EF-hand loop domains can be swapped into further extending the lanthanide metal binding capacity of this chassis.

EXPANDING THE FUNCTIONALITY OF MATERIALS USING SOLVENT-FREE PROTEIN LIQUIDS

Authors:
Joseph M. Slocik, Claretta J. Sullivan, Caitlin Bojanowski, Nancy Kelley-Loughnane, Rajesh R. Naik, and Patrick B. Dennis

Biology offers an unparalleled level of control over material synthesis and assembly, unlimited functionality, and a wealth of exquisite inorganic and biomolecular structures. However, biological systems rapidly denature and lose activity at high temperatures, require aqueous conditions to maintain stability, and generally possess short shelf-lives. Consequently, the creation of solvent-free protein liquids has the potential to transform the application landscape of biological materials by overcoming the inherent deficiencies in biology. For example, protein liquids have greatly increased the thermal tolerance of proteins to high temperatures
imparted new solubility in non-aqueous and biologically incompatible solvents, and enabled the temporary modification of bacterial phenotypes. In total, we will present the multi-functional nature of protein liquids by highlighting their extraordinary thermal stability ability to transfect, modify, and stabilize microorganisms with exogenous proteins
and miscibility and integration with a wide range of solvents and polymeric based materials. Notably, solvent-free protein liquids provide new capabilities and opportunities that are not presently accessible in biology (eg. high temperature processing and compatibility with organic solvents).

ELECTROCHEMICALLY CONTROLLED NEUROTRANSMITTER DELIVERY FOR *IN VITRO* NEUROMODULATION

Authors:
Craig Milroy[1,2], Tyson Back[3], Nick Glavin[3], Steve Kim[1]
Affiliations:
[1] 711th Human Performance Wing, Air Force Research Laboratory (AFRL), Wright-Patterson Air Force Base (WPAFB), OH 45433, USA
[2] National Research Council (NRC) Associateship Program, National Academies of Science, Engineering, and Medicine, Washington, DC 20001, USA
[3] Materials and Manufacturing Directorate, Air Force Research Laboratory (AFRL), Wright-Patterson Air Force Base (WPAFB), OH 45433, USA

The National Academies of Science and Engineering have issued a grand challenge to “reverse-engineer the brain”. Addressing this challenge requires a more complete understanding of how connections between individual neurons and groups of neurons are formed and maintained, as well as a more thorough elucidation of the signaling processes that drive neural network optimization. However, to achieve these goals, it is first necessary to develop the appropriate fundamental tools and protocols for adaptive biointerfacing, bottom-up neuroscience, and neuromodulation, which all involve a tightly coordinated synergy between sensors and specialized devices for neurotransmitter delivery. To date, extensive research efforts have been devoted to neurotransmitter sensing, but relatively little attention has been given to developing methods for electronically controlled *in vitro* neurotransmitter delivery. To address this challenge, our research team has developed devices that can both sense and selectively dispense the biochemical cues that drive the formation, regulation, and modulation of *in vitro* cellular/neural networks. Here, we report electrochemically controlled dispensing of both excitatory and inhibitory neurotransmitters (*e.g.* glutamate, GABA, dopamine) from functionalized electrodes that operate at low voltage. The regulated uptake and release of neurotransmitters is mediated by electrochemically controlling the electrostatic affinity between the neurotransmitter and a polarized electrode that has been functionalized with an electroactive affinity

molecule. To optimize our devices, our team tested a variety of electrode functionalization approaches (*e.g*. alkane-thiol or silane chemistry, layer-by-layer or covalent modification) and a variety of electroactive affinity molecules (*e.g.* ferrocene, methylene blue, viologens). Our controlled dispensing devices function as a faradaic capacitor – when the functionalized electrode is anodically polarized, the redox affinity molecule becomes oxidized (*i.e.* positively charged), and the neurotransmitter loads on the functionalized electrode through electrostatic attraction to the oxidized redox affinity molecule. The neurotransmitter may be conveniently "stored" on the electrode by maintaining the applied electrode potential, and then released on demand by reversing the applied bias using controlled steps or pulses, which reduces the electroactive affinity molecule and releases the neurotransmitter. This controlled adjustment of applied electrode potential permits quantitative biochemical release. In addition, the electrochemical signature associated with the loading of different neurotransmitters permits sensing as well as delivery.

We confirm the operational principle through a variety of analytical and electroanalytical techniques, such as cyclic voltammetry, chronopotentiometry, open-circuit potentiometry, continuous enzymatic monitoring, electrochemical quartz crystal microbalance (EQCM), X-ray photoelectron spectroscopy, and triple quadrupole mass spectrometry. We then demonstrate that the functionalized electrodes may also be coupled with electrochemical transistors (ECT) for triggered neurotransmitter release in conjunction with changes in the concentration of important physiological ions (*i.e.* sodium, potassium, calcium) or biochemical messengers. Finally, we will present the steps we have taken to implement these devices within organ-on-a-chip systems, and demonstrate the utility of our devices for studying the formation and longitudinal evolution of neural networks within *in vitro* neuronal colonies.

Keywords: Neuromodulation, electrochemistry, neurotransmitters

MUSKOX KERATINIZED ELECTROSPUN NYLON NANOFIBERS AS NEXT-GENERATION EXTREME COLD WEATHER TEXTILES

Authors:

Kathryn Cordiero[1,2], Victor Roman[3,4], Evan Delisle[3], Patrick Dennis[3], Blake Stamps[3], Nancy Kelley-Loughnane[3], M. Tyler Nelson[1]

Affiliations:

[1] 711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson AFB, OH 45433; [2] University of California-Los Angeles, Los Angeles, CA 90095; [3] Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH 45433; [4] UES – Eqlipse Technologies Inc., Dayton, OH 45432

As the U.S. military projects force in a variety of regions with a vast range of temperature and humidity conditions, effective thermal insulation will be key to protecting sensitive infrastructure assets and personnel. Looking at current cold weather gear, there is an obvious need for improvements to meet the harsh conditions of the Arctic. Muskoxen, a large woolly-looking mammal, survive in the some of the most uninhabitable regions on Earth, the Arctic. Temperatures in the Arctic can get as low as -80 F, yet these large mammals are able to survive due in part to their unique inner coat called, Qiviut. The average fiber diameter (AFD) of Muskox qiviut fibers is 17 µm in diameter, nearly 10 µm smaller than common Merino sheep wool fibers. The unique size and packing efficiency of the qiviut is thought to provide enhanced thermal insulating properties and mechanical strength over sheep wool. While cultivation of sheep for wool production is a feasible venture, Muskox are an endangered species with limited numbers, making natural cultivation of qiviut not feasible to meet

the supply chain demands of U.S. forces. Therefore, we derived a synthetic biology approach, leveraging the power of microbes, to biomanufacture the unique keratin peptides that comprise Muskox qiviut fibers. The syn-bio muskox keratin peptides are then elegantly integrated into a biomimetic electrospun nylon composite-nanofiber textile in support of Warfighter protection and performance in Arctic climates. Using microbes to produce the Muskox keratin peptides allows for rapid scalability, U.S. maintained supply chains, and next-generation textiles for supporting not only the larger U.S. DOD, but also NATO demands for improved cold weather gear. Furthermore, natural resources like Merino wool, Down goose feathers, and cashmere is a very expensive venture and poses both an economical and environmental concern for long-term sustainment. To date, we have developed new methods to extract muskox keratins, incorporate them with common Nylon synthetic polymers, and electrospun ultra-fine composite nanofibers with enhanced insulative properties. We have further optimized this process to limit the amount of keratin needed to obtain a significant improvement in insulative properties, by developing a core-shell nanofiber production approach. Lastly, we developed methods to create hollow core fibers, creating an insulative air-gap, further enhancing and biomimicking the structure of muskoxen fibers. Utilizing this synthetic-biology approach we have developed a novel cold weather textile aimed at sustainably providing next-generation textiles for extreme environments.

CHARACTERIZATION OF WOOL FIBERS FROM ARCTIC ANIMALS: BIOTECH SOLUTIONS FOR KERATIN-INFUSED TEXTILES

Authors:
Victor A. Roman[1,2], Logan Cummings[1,2], Blake W. Stamps[1] & Nancy Kelley-Loughnane[1]
Affiliations:
[1] Soft Matter Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio, USA
[2] UES, Inc., Dayton, Ohio, USA

Efficient thermal protection is a necessity for military personnel deployed in cold weather operations. Sheep wool is a highly prized, keratin-rich, insulating material. The inner coat of muskox (*Ovibos moschatus*) and its keratin-rich fibers, commonly known as *qiviut*, has been reported by commercial vendors as being stronger, softer, and warmer than sheep wool. In this work, we aimed to characterize the thermal and mechanical properties of the naturally occurring wool fibers of muskox and merino sheep wools, to gain insights into new thermal insulating resources. Thermal characterization of muskox qiviut and merino sheep wools showed no significant differences in thermal conductivity values. Interestingly, whole protein samples extracted from qiviut and merino wools, retained similar insulating potential to the one observed with the wools, suggesting that the proteins present in these fibers could be enough to confer thermally insulating properties to other materials. Thus, protein contents alone might be sufficient to achieve insulating properties. Moreover, single-fiber tensile strength testing, using a dynamic mechanical analyzer of commercially obtained qiviut and merino ultrafine wool, showed that muskox fibers are stronger than merino ultrafine wools. These findings indicated that qiviut fibers are mechanically more robust since they require larger force loads to fracture when compared to merino sheep fibers. Better understanding of the protein content within these fibers, especially which exact keratins are composing these fibers, will open new avenues for using a synthetic biology approach to produce keratins and study their incorporation into new biomaterials for the textile industry. Finally, this work will explore the

combination of well-studied textile polymers with keratin material and their effect its thermal and mechanical properties.

RAPAMYCIN INDUCTION INCREASES CUTINASE GENE EXPRESSION AND SECRETION VIA THE MTOR PATHWAY IN *P. LAURENTII*

Authors:
Stephen Zelik [1], Blake Stamps [2], Pat Dennis [2], Victor Roman [1], Nancy Kelley-Loughnane [2], Justin C. Biffinger [3], Daniel Barlow [4], Qin Lu [4],
Affiliations:
1: UES, Inc., Dayton, Ohio, USA, 2: RXEB, Air Force Research Laboratory, Wright-Patterson AFB, Ohio 3: Chemistry Department, University of Dayton, 300 College Park, Dayton, OH. 45469
4: Chemistry Division, US Naval Research Laboratory, Washington, DC

Resource reutilization is a growing field of study that includes the intentional degradation of plastic polymers. One polymer, polyurethane, is degraded via the activity of cutinase in the fungus *Papiliotrema laurentii* although when other more preferential carbon substrates such as glucose are available, its' activity is repressed. Cutinase activity can be induced via the mTOR pathway in Eukaryotes. mTOR is also associated with autophagy, a highly conserved condition in eukaryotes that recycle cellular components in times of nutrient deprivation. We hypothesized that by treating *P. laurentii* with rapamycin cutinase expression would be enhanced. Further, we predicted that polymer degradation would be correspondingly enhanced even in the presence of otherwise preferred carbon substrates such as glucose. Our data show that rapamycin upregulates cutinase expression in *P. laurentii* even in the presence of glucose, confirming our hypothesis and prediction. By fully understanding the links between the mTOR pathway, autophagy, and polymer degradation in fungi, we may be able to greatly increase our ability to selectively degrade plastics and enhance our ability to control resource reutilization processes at an industrial scale.

Other Technical Presentations

ARPA-H: THE MISSION

Authors:
Dr. Andrew Kilianski

Abstract not available at this time.

AFOSR TECH TALK: MATERIALS DISCOVERY THROUGH EXPLORATION OF BIOMOLECULAR SPACE

Author:
Dr. Rein V. Ulijn

Living systems provide the most sophisticated materials known. These materials and systems are

created from a fully conserved set of just a few dozen building blocks common to all life forms. This observation begs a profound question: why can't everything, including things that life has not explored, be made from biological building blocks? The Ulijn lab is taking steps to making this vision a reality, not by copying biology, but by developing methodology for bottom-up design, discovery and evolution of functional materials and biofluids to redesign biomolecules for a variety of applications.1 The talk will include our latest research on ongoing projects: (i) Design of peptide modalities that give rise to formation of liquid condensates;2,3 (ii) Mechano-responsive peptide crystals;4 (iii) Drug-matched peptide nanoparticles; (iv) Experimental learning and memory using sequence-adaptive peptide mixtures.5,6 Overall, our research demonstrates that peptides, and dynamically exchanging mixtures of peptides, show significant potential as designable and tunable nanomaterials for a variety of applications in biomedicine and green nanotechnology.

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AFOSR TECH TALK: SYNTHETIC MUCUS BIOMATERIALS

Author:
Dr. Adam Braunschweig

Animal mucuses are currently being investigated for diverse applications, including cosmetics, wound-healing, drug-delivery, and as adhesives. However, challenges related to batch-to-batch variability, stability, and contaminants as well as the limited knowlegde of the composition of these materials has precluded their adoption for these proposed applications. Ideally, synthetic materials could be produced that reproduce the advantageous properties of natural mucus, and also provide the additional benefits of homogeneity, stability, and tailorability. Here, we describe the synthesis, formulation, and properties of synthetic mucin gels that are designed to emulate the structure and properties of their natural counterparts. This talk will describe in detail the composition of snail mucus, the multistep synthesis of novel synthetic snail mucin polymers, and their rheological and mechanical behavior, including work of adhesion and elastic modulus. In doing so, it will be shown that synthetic polymers can recaptiulate the advantageous properties of natural mucuses.

ADVANCING MICROPHYSIOLOGICAL SYSTEMS FOR HOLISTIC WARFIGHTER ASSESSMENT

Authors:
M. Tyler Nelson[1], Matthew W. Grogg[1], Eric J. Reed[1,2], Eric Romer[3], Patrick McLendon[1,4], Krysten Jones[1,4], Rachel Hopton[1,4], Taylor Chermak[1,5], Caleb Anthony[1,4], Amanda Hanes[1,4], Mary E. Huddleston[1,4], Katherine Ingram[1,4], Raquel Moore[1,4], Salvi Kambarova[1,2]
Affiliations:
[1] Air Force Research Laboratory, 711th Human Performance Wing, Wright-Patterson AFB, OH 45433; [2] Wright State University, Fairborn, OH 45432; [3] Naval Aeromedical Research Unit – Dayton, Wright-Patterson AFB, OH 45433; [4] UES, Inc., Dayton, OH 45413; [5] The Ohio State University, Department of Microbiology, Columbus, OH 43201

This study pioneers the development of diverse organ-on-a-chip platforms within microphysiological systems (MPS) to comprehensively evaluate Warfighter responses to environmental and operational stresses. Leveraging microfluidic-based technologies, we have crafted distinct brain, gut, lung, adipose, and muscle organ-on-a-chip modules, each faithfully replicating the microenvironment of its corresponding organ. Next-generation sequencing, metabolomics, microelectrode array (MEA), and advanced imaging tools form the backbone of our methodology. Exposing these microphysiological systems to a range of stressors, including toxicological challenges, high-altitude impacts, physical, and cognitive fatigue, allows us to uncover organ-specific responses, providing a foundation for future investigations into interconnected tissue dynamics.

Our modular approach allows for individual organ assessments while setting the stage for future integration, emphasizing the interconnected nature of these organ-on-a-chip platforms. Currently, we focus on organ-specific stress responses, but our vision is to explore the collective impact and systemic interactions between these distinct modules. The integration of next-generation sequencing and metabolomics uncovers molecular signatures associated with stress-induced responses. Simultaneously, MEA technology enables real-time monitoring of electrical activities, complemented by advanced imaging tools capturing dynamic changes in cellular morphology and function.

This forward-looking research not only contributes to the evolution of personalized medicine and military healthcare but also addresses the complex interplay of physiological responses in diverse operational conditions. The modularity of our approach positions us at the forefront of biomedical engineering, paving the way for a more holistic understanding of stress responses and their implications for military readiness and performance optimization. As we continue to refine and integrate our organ-on-a-chip platforms, this study signifies a crucial step toward a comprehensive understanding of Warfighter responses to environmental stresses, laying the groundwork for advancements in military health and operational readiness.

DISCLAIMER: The views expressed are my own and do not reflect the official policy or position of the Department of the Air Force, Department of Defense, or the U.S. Government.

BIOTECHNOLOGICAL PRODUCTION OF PYOCHELIN SIDEROPHORE IN RARE EARTH ELEMENT ENRICHMENT

Authors:
Adam M. Reed[1], Amanda L. Barry Schroeder[1], Oscar N. Ruiz[2], Chia-Suei Hung[2], Andrea Hoffmann[1] and Kara L. Martin[3]

Affiliations:
University of Dayton Research Institute, Dayton, OH
RXEB, Biomaterials Branch, Photonic, Electronic & Soft Materials Division, Materials & Manufacturing Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH
RQTF, Fuels & Energy, Turbine Engine Division, Aerospace Systems Directorate, Air Force Research Laboratory, WPAFB, Dayton, OH

POC Email: adam.reed.16.ctr@us.af.mil

Low-cost storage technologies and more efficient engines are critical for energy generation from renewable sources. Rare earth elements (REE) have been identified as resources needed for battery energy storage units, consumer electronics and electric vehicles. The harvest and recycling of rare elements has become of increasing demand for use in military and aerospace applications such as energy-saving lamps, lasers, radar, computer drives, electric motors, and as catalytic converters in industrial applications. This study is thought to develop a novel methodology that uses the ion chelator Pyochelin as an affinity compound for purification of lanthanide REEs such as Nd, La, Tb, Dy, Yb, Eu.
Key words: pyochelin, siderophore, rare earth elements.