RUTGERS

RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY

BIOTECHNOLOGY TRAINING PROGRAM

ANNUAL REPORT
2019

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Rutgers, The State University of New Jersey
BIOTECHNOLOGY TRAINING PROGRAM

The PhD Training Program in Biotechnology at Rutgers, The State University of New Jersey was established in 1989. It is one of the select group of such programs throughout the country funded by the National Institute of Health (NIH). The 2018-19 year marks the 29th year of NIH funding. Biotech Fellows are supported for two years through the funding provided by the NIH and matched by the University. For the 2019-2020 year, the NIH is providing 10 fellow positions and the University is providing an additional 3 positions, 2 from RBHS.

The aim of the program is to train a new breed of creative investigators who are able to translate basic science discoveries into technological developments for the needs of society, government, and industry. Students in the program become: (1) well educated within a single biotechnology-related discipline (e.g. biochemistry, chemical engineering, molecular biology), and (2) fluent in the language, approaches and principles of the biological and physical sciences, in general.

The research programs of the training faculty address a broad spectrum of problems in biotechnology. The majority of the individual and collaborative projects fall within two major interdisciplinary research thrusts:

Genomics, Proteomics, and Structural Biology: The past few decades have seen great technical advances in molecular and cell biology that have led to the development of new therapeutics and diagnostics which will have a profound impact on medicine for years to come. With the Human Genome Project complete, a massive effort is being undertaken to build from the molecular level in a step-wise fashion all the way to complex behavior and function. This effort will require further discovery and analysis of biological systems together with integration of high throughput and genetic manipulation technologies in experimental biology, sophisticated data management and statistical analysis techniques from mathematics and computer science, and systems modeling and fabrication tools from engineering. Every major pharmaceutical company is currently invested heavily in "post-genome" technologies, and numerous biotechnology companies have been created in areas such as genomics, proteomics, and systems biology. Genomics-based products and technologies are estimated to exceed $50 billion.

Tissue Engineering, Regenerative Medicine, and Drug Delivery: Without question, one of the most fertile biotechnological areas for the development of new and innovative medical therapies for the next century lies in the realm of regenerative medicine and tissue engineering. Given the remarkable advances in fundamental understanding of the functions and behaviors of cells and tissues over the past few decades, we are poised in the beginning of the 21st century to translate this basic knowledge into vast improvements in the practice of medicine. By combining basic science, engineering problem-solving and clinical wisdom, age-old handicaps that used to devastate people's lives - blindness, deafness, paraplegia, organ dysfunction and failure, memory loss, and even death - may be circumvented by cell transplants, advanced drug delivery systems, intelligent prostheses, neural implants, artificial organs, and natural organs re-grown after injury or disease. In addition to the latter, we foresee that cell and tissue-based integrated systems will, in the not-too-distant-future, become

pharmaceutical industry standards for early and late stages of drug discovery and drug testing, in the same manner that combinatorial approaches have revolutionized early steps of drug synthesis and discovery. Finally, the NIH estimates that the current world market for replacement organ therapies is in excess of $350 billion, and the projected U.S. market for regenerative medicine is estimated at $100 billion.

Program Faculty

Training faculty, their department affiliation, and their research interests are provided in **Appendix A.** The individuals listed have been selected on the basis of their research expertise, proven ability to engage in collaborative, interdisciplinary work, national and international scientific reputations, proven ability to attract continuing external research support, and established records of didactic and research training in biotechnology. The primary roles of the members of the biotechnology training faculty are to: 1) contribute to the teaching mission of the program, 2) direct the research of individual trainees, 3) serve on thesis committees of individual trainees, and 4) serve as needed on program committees.

Trainee Candidates

Only students of exceptional abilities and motivation are admitted to the Biotechnology Training Program. The program is aimed at producing the very best students in the field. Selection is based on academic performance and potential for future excellence. Students must first gain admission to one of the Ph.D. granting programs with which the training faculty are affiliated. Admission to the Biotechnology Training Program is determined by the Biotechnology Program Admissions Committee. The trainees are expected to meet the same criteria required of graduate students awarded the most competitive awards, such as NSF Graduate or Rutgers Presidential Fellowships. These include an outstanding scholastic record as measured by undergraduate cumulative averages, Graduate Record Examination scores, previous research experience, letters of recommendation, and an indication of leadership potential. Interviews are conducted with all students.

Some students apply to the program after a year or two of study. These students petition their graduate program directors to submit applications to the Biotechnology Training Program on their behalf, and are interviewed if deemed suitable. If accepted, these students are expected to fulfill all requirements of the program, including the coursework and industrial laboratory rotations. No student, regardless of his/her year of admission, is supported longer than two years by the program. Biotech fellows are listed in **Appendix B.**

Student Research, Publications, and Presentations

A listing of current research, publications, and presentations of our trainees is provided in **Appendix C**. For the past year alone, over 40 papers and presentations have been made by Biotechnology Program students. This successful publication and presentation history certainly supports the fact that we continue to train highly skilled and effective scientists and engineers who will contribute to the advancement and success of biotechnology.

Summer Industrial Internship Program - Appendix D

The purpose of this program is to provide an opportunity for the students to gain access to industrial facilities and become more aware of the "gestalt" and practice of industrial research and development. At a minimum, students spend eight weeks full time at an industrial site under the guidance of a particular industrial investigator. These experiences may, on occasion, lead to the involvement of an industrial mentor on the student's dissertation committee. Students who have prior extensive industrial experience may elect to opt out of this requirement; but many of these students still wish to do rotations in different fields. We are extremely fortunate to have a tremendous variety of experiences available.

Courses – Appendix E

Biotechnology Program Specific Courses: The Biotechnology Training Program specific courses and other activities that form a core experience provide the student with a perspective on biotechnology from multiple vantage points: 1) the advanced academic research viewpoint (the Topics in Advanced Biotechnology Course and Academic Lab Rotations), 2) the traditional Biotechnology Industry viewpoint (the Bioengineering in the Biotechnology and Pharmaceutical Industries course and the Industrial Internship), and 3) the start-up and new venture viewpoint (the Innovation and Entrepreneurship for Science and Technology course and the Industrial Internship)

Topics in Advanced Biotechnology I: After the Biotech Program fall orientation which takes place the last week in August, students and faculty meet biweekly during the fall semester for the Topics course. This forum introduces the new students to research opportunities within the program and allows advanced students to sharpen their presentation skills by providing an experienced audience to critique their work. Students who do not have ongoing work to describe may present a recent paper from the literature which is chosen in consultation with the faculty/student group.

Topics in Advanced Biotechnology II: This course is one of the primary unifying threads of the Program. It occurs biweekly during each spring semester (for 2-3 hour sessions), and all students in the training program (those currently supported as well as those who were supported in the past) are required to attend. The course serves as a forum to: 1) highlight and unify ongoing biotechnology research on campus, 2) introduce emerging new areas of biotechnology to students and faculty, and 3) provide trainees with insight into the technological development of basic discoveries. Faculty guide students in the choice of literature articles that they will present. Critical analysis of data, its interpretation and implications are highlighted, and special attention is paid to applied research, technology-oriented issues, ethical considerations, and policy-oriented issues in the subject area. In this regard, invited investigators from industry play a key role. By having students enroll in the course during their entire graduate career (every spring semester), it is possible to involve advanced students in the selection of topics and seminar speakers (including the responsibility for organizing speakers) and to encourage their interaction with scientists from outside institutions.

Innovation and Entrepreneurship for Science and Technology: This course introduces and outlines the fundamentals of "technology entrepreneurship" and introduces a framework for identification of high-potential, technology-intensive, commercial opportunities, gathering required resources (human and

financial), and maturing the innovation to a commercializable product. The course places a specific focus on commercialization derived from scientific and technological research with special emphasis on biotechnology and the life science industry. The course is led by Susan Engelhardt and Martin Yarmush with guest lecturers from industry and academia. The course objective is to have students complete the class with: 1) an understanding of the major components of the life cycle from research to innovation to commercialization, 2) knowledge of the many ways that innovation manifests itself, in the context of start-up, corporate, social and public sector concerns, 3) practical methods to intelligently and objectively evaluate potential commercialization opportunities, and 4) a framework within which to consider the ethical issues that are intertwined with entrepreneurial activities. Through the collection of lectures and projects, students build upon the following critical skills for entrepreneurial success: 1) opportunity evaluation, 2) strategic thinking, 3) teamwork, 4) art of selling, persuasion and motivation, oral and written communication, basics of start-up legal concepts, basics of startup finance and accounting. This course was developed in response to student demand.

Bioengineering in the Biotechnology and Pharmaceutical Industries: The goal of this course is to offer students insight into the practical aspects of industrial bioprocessing. Industrial practitioners from various fields of expertise provide lectures and facilitate discussions highlighting problems and issues that engineers and scientists encounter. Topics vary from year to year but always include: drug discovery, drug metabolism, microbial fermentation and mammalian cell culture optimization and scale-up, monoclonal antibody, vaccine and gene therapy production, downstream purification, drug delivery, formulation, regenerative medicine, stem cell culture, tissue engineering, cellular therapies, regulatory considerations, manufacturing challenges, and clinical research. This course provides students with exposure to topics which are beyond the scope of a purely theoretically-structured course. After taking this course, students have a much better understanding of the challenges that engineers and scientists face in industrial bioprocessing.

Interdisciplinary Biostatistics Research Training For Molecular And Cellular Sciences: Enhancing Rigor And Reproducibility: This course will provide students with a strong foundation in statistical approaches to data analysis and will be specifically tailored to the molecular, cellular, and tissue biotechnology and bioengineering data relevant to their thesis projects. Two particularly important components of the course involve the training of students on how to: 1) critically assess and interpret published scientific data, and 2) enhance and optimize experimental rigor and reproducibility. An active learning strategy combining didactic instruction and experiential training will reinforce understanding and appreciation for the importance of data analysis in designing rigorous and reproducible data suitable for publication in top-tier scientific journals. This course will be taught by bench scientists with a solid grasp of statistical methodology, using easy to understand terminology, and who are very effective teachers of statistics to wide audiences.

Professional Preparedness in Biotechnology: Although current courses in the typical graduate curriculum appropriately deliver strategic discipline-based learning for life science and engineering graduate students, the broader biotech and health science industry further demands that scientists be prepared to serve a variety of distinct functions within the life and biomedical sciences ecosystem, and to understand broader developmental aspects of the business of science and engineering in a professional environment. Many scientific professionals, while experts in their respective fields, have little academic/professional background in business management; skills that ensure that scientific projects and research are implementable, feasible and sustainable. In addition, these skills work to

expand scientists' and researchers' professional reach and help them to realize their true career potential. This course entitled, "Professional Preparedness in Biotechnology" will enhance students' competitive skills and introduce additional layers of specialized competence enabling immediate contribution within diverse organizations in the life and biomedical sciences commercial sector. Students will develop business, communication, management, (and other), and skills.

Applications In Medical Device Development: This course will provide students insight into the application of a variety of medical devices, and introduce business concepts as they relate to medical devices from a realistic industrial perspective. Representative fields including but not limited to cardiovascular, orthopedics, diagnostics, imaging, rehabilitation, and dental will be covered. Industrial practitioners provide lectures and facilitate discussions highlighting problems such as manufacturing issues or project management challenges that engineers and scientists may encounter when dealing with the medical device industry.

Symposium and Orientation – Appendix F

The Biotechnology Program's Annual Minisymposium of faculty, trainees, and industrial investigators serves as a forum for presentation and review. The meeting is a one-day offsite retreat and colloquium, during late summer, where the trainees, faculty, and industrial members present research papers and posters. The meeting also helps to introduce new students to the research programs at Rutgers and to other topics of interest to the biotechnology industry. Speakers from industry have discussed health care reform and entrepreneurship, for example, at past retreats. Student awards are presented to the top research posters.

Alumni- Appendix G

Since its inception, the Biotechnology Training Program has trained over 150 PhD candidates. These graduate students have gone on to achieve successful careers in both industry and academia. Many of our alumni currently support our program by hosting our trainees as interns for the summer and by participating in Biotechnology Training Program courses.

APPENDIX A: BIOTECHNOLOGY TRAINING PROGRAM FACULTY

Faculty	Role in Program	Research Interest
Alder, Janet, PhD Associate Professor Neuroscience and Cell Biology	Mentor	Neuronal Development Traumatic Brain Injury
Androulakis, Ioannis, PhD Professor Biomedical Engineering	Mentor	Quantitative Systems Biology
Arnold, Edward, PhD Distinguished Professor Chemistry and Chemical Biology	Mentor	Structural Biology of Polymerases, HIV
Berthiaume, Francois, PhD Professor Biomedical Engineering	**Executive Committee**	Tissue Engineering, Metabolic Engineering
Blaser, Martin, MD, PhD Henry Rutgers Chair Professor Center for Advanced Biotechnology and Medicine	Mentor	Cancer, Genomics, Immunology, Microbiome
Brzustowicz, Linda, PhD Distinguished Professor Genetics	Mentor	Molecular and Statistical Genetics in Neuroscience
Bunting, Sam, PhD Assistant Professor Molecular Biology	Mentor	DNA Repair
Burley, Stephen, MD, PhD Henry Rutgers Chair Professor Chemistry and Chemical Biology	Mentor	Data Sci, Oncology, Drug Discovery, Bioinformatics, Antibiotic Resistance
Cai, Li, PhD Associate Professor Biomedical Engineering	Mentor	Neural Stem Cells in Development & Disease
Copeland, Paul, PhD Professor Molecular Biology	Mentor	Regulation of Gene Expression: Translation
Driscoll, Monica, PhD Professor Molecular Biology	Mentor	Molecular Genetics of Aging and Neurodegeneration
Dunn, Michael, PhD Professor Orthopaedic Surgery	Mentor	Musculoskeletal Tissue Engineering
Ebright, Richard, PhD Board of Governors Prof Chemistry and Chemical Biology	Mentor	Antibacterial Drug Discovery, Transcription
Firestein, Bonnie, PhD Professor Cell Biology and Neuroscience	Mentor	Neurodevelopment, TBI, SCI, Schizophrenia
Freeman, Joseph, PhD Associate Professor Biomedical Engineering	Mentor	Musculoskeletal Tissue Engineering
Gormley, Adam, PhD Assistant Professor Biomedical Engineering	Mentor	Bioinspired Nanomaterials

Faculty	Role in Program	Research Interest
Grumet, Martin, PhD Professor Cell Biology and Neuroscience	**Executive Committee**	Neural Stem Cells in Development & Disease
Jacinto, Estella, PhD Associate Professor Molecular Biology	Mentor	Growth Control, Cancer, Immunology
Jiang, Peng, PhD Assistant Professor Cell Biology and Neuroscience	Mentor	Stem Cell Regenerative Medicine
Jin, Victor, PhD Associate Professor Pharmacology	Mentor	Investigational Drug Dev., Oncology, Met & Genetic Diseases
Khare, Sagar, PhD Assistant Professor Chemistry and Chemical Biology	Mentor	Protein Design & Engineering
Lee, Ki Bum, PhD Professor Chemistry and Chemical Biology	**Executive Committee**	Nanomaterials for Biomedical Applications
Lobel, Peter, PhD Professor Molecular Biology	**Executive Committee**	Lysosomal Storage Diseases, Proteomics
Madura, Kiran, PhD Professor Pharmacology	Mentor	Ubiquitin-Mediated Protein Degradation in DNA Repair & Signal Transduction
Millonig, James, PhD Associate Professor Neuroscience & Cell Biology	Mentor	Neurodevelopmental & Autism Spectrum Disorders
Moghe, Prabhas, PhD Distinguished Professor Biomedical Engineering	Mentor	Nanomedicine, Stem Cell Bioengineering
Nanda, Vikas, PhD Associate Professor Biochemistry	Mentor	Protein Engineering, Biomaterials, Drug Design, Bioenergetics
Olabisi, Ronke, PhD Assistant Professor Biomedical Engineering	Mentor	Tissue Engineering, Regenerative Medicine
Pang, Zhiping, PhD Assistant Professor Neuroscience and Cell Biology	Mentor	Molecular Mechanisms of Synaptic Dysfunction
Parekkadan, Biju, PhD Associate Professor Biomedical Engineering	Mentor	Cell Engineering
Roth, Charles, PhD Professor Biomedical Engineering	**Executive Committee**	Nanomedicine
Shreiber, David, PhD Professor Biomedical Engineering	Mentor	Biomech/Biomat, Regen. Med., Drug Delivery
Sinko, Patrick, PhD Parke-Davis Chair Professor Pharmaceutical Sciences	Mentor	Nano & Microscale Drug Delivery

Faculty	Role in Program	Research Interest
Soto, Martha Associate Professor Pathology	**Mentor**	Cell Polarity, Embryonic Morphogenesis, C.elegans, Development Genetics
Stock, Ann, PhD Professor Molecular Biology	**Co-Director**	Structure/Function Analysis of Signal Transduction Proteins
Sy, Jay Assistant Professor Biomedical Engineering	Mentor	Drug Delivery, Biomaterials
Vazquez, Maribel, PhD Associate Professor Biomedical Engineering	Mentor	BioMEMS, Reg Med, Retinal Transplantation
White, Eileen, PhD Professor Molecular Biology	Mentor	Apoptosis & Autophagy in Cancer
Williams, Lawrence, PhD Professor Chemistry and Chemical Biology	Mentor	Molecular Structure and Reactivity, Protein Modeling.
Yarmush, Martin, MD, PhD Monroe Chair Professor Biomedical Engineering	**Director**	Tissue Engineering, NioMEMS, Stem Cells, Medical Devices
Zahn, Jeffrey, PhD Professor Biomedical Engineering	Mentor	BioMEMS, Point of Care, Neurotechnology
Zaratiegui, Mikel, PhD Assistant Professor Molecular Biology	Mentor	Chromatin, Replication Transposons

APPENDIX B: BIOTECHNOLOGY TRAINING PROGRAM FELLOWS

FELLOWS	THESIS TITLE/CURRENT RESEARCH
Acevedo, Alison BME Androulakis	Evaluation of Complex Network Effects of Methylprednisolone in Multiple Tissues
Alarcon, Yoliem Miranda BME Shreiber	A Thermoreversible and Photoactive Collagen-Based Scaffold for Tissue Engineering Applications
Anderson, Jeremy BME Cai	Endogenous Neural Stem Cell Activation after Traumatic Brain Injury
Boreland, Andrew CBN Pang/Jiang	A Novel Human Brain Organoid Model for HIV-Infection
Burr, Alexandra BME Parekkadan	Circadian Response Element Driven Therapeutic Secretion Mediated by Gene Therapy
Cheng, Larry Pharm Tian	Alternative polyadenylation in the placenta
Chuang, Skylar CCB Lee	Enhancing Efferocytosis Through Chimeric Antigen Receptor Macrophage Combined with Nanoparticle Reprogamming
Davis, Mollie BME Yarmush	An Integrated Anesthetic-MSC Therapeutic for Osteoarthritis
DiMartini, Emily BME Shreiber	Targeted Delivery of Therapeutic Factors via Free Radical Mediated Immobilization
Fritz, Zachary BME Yarmush	Improving Cancer Diagnostics with Protein Energetics Modeling and Microfluidics
Godesky, Madison BME Shreiber	Next Generation Hyaluronic Acid-Based Bioinks for Cell-Friendly 3D Printing
Guasp, Ryan CDB Driscoll	Elucidating the molecular mechanism of the exopher, a novel extracellular vesicle
Krzyszczyk, Paulina BME Berthiaume	Investigating the Effects of Hemoglobin-Based Complexes on Macrophages for Use in Chronic Wounds
Leipheimer, Josh BME Yarmush	A Hand-held, Portable and Low Cost Robotic Device for Autonomous Intravenous Access
Luo, Jeffrey CCB Lee	Hybrid Magnetic Nanoparticle-Nanofiber Substrate for Stem Cell Behavior Control

FELLOWS	THESIS TITLE/CURRENT RESEARCH
Marrero, Ileana BME Yarmush	Alginate-Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment
Melentijevic, Ilija MBB Driscoll	Exophers: A Novel Neuronal Debris Clearance Pathway
Newman, Jenna MBS Zloza	Utilizing the seasonal influenza vaccine as a treatment for cancer
Newton, Brandon BME Freeman	Characterization and Optimization of a Positively Charged Poly(Ethylene Glycol) Diacrylate Hydrogel as an Actuating Muscle Tissue Engineering Scaffold
Okeke, Evelyn BMB Madura	Characterization of the nucleocytoplasmic trafficking of Rad23, a shuttle factor that functions in protein degradation
Omelchenko, Anton Neuroscience Firestein	Exosome-based Delivery of RNAi Therapeutics to Target Traumatic Brain Injury
Patel, Misaal BME Cai	Therapeutic role of Gsx1 in neurogenesis and astrogliosis after spinal cord injury.
Perez, Xiomara BME Yarmush	Mesenchymal Stromal Cells and Therapeutic Nanoparticles for the Treatment of Traumatic Brain Injury
Pfaff, William BME Dunn	Laterally Anisotropic Composite Scaffold Designed for Regeneration of Articular Cartilage of the Knee
Rathnam, Chris Chemistry Lee	NanoScript: A Synthetic Transcription Factor for Regenerative Medicine
Reilly, Eve MBS Zaratiegui	Identifying factors involved in coordination of heterochromatin inheritance and DNA replication
Singh, Nisha CBN Firestein	Role of Cypin Inhibition After Spinal Cord Injury
Tan, Victor Pharm Jin	Phosphoproteomic contributions towards resistance of advanced prostate cancer against androgen deprivation therapies
Turk, Liam Biochem Comoletti	Solving the structural activation mechanism of the reelin pathway and expanding reelin's interaction profile
Upadhya, Rahul BME Gormley	PET-RAFT and SAXS: High Throughput Tools to Study Compactness and Flexibility of Single Chain Polymer Nanoparticles
Wood, Caroline BME Sy	Improving Drug Pharmacokinetics in the Brain Using Cerebral Spinal Fluid Modulating Polyanhydride Systems

APPENDIX C: CURRENT RESEARCH, PRESENTATIONS, PAPERS, AND PATENTS

ALISON ACEVEDO
Advisor: Ioannis Androulakis

Synthetic corticosteroids, such as the corticosteroid methylprednisolone (MPL), are widely used anti-inflammatory and immunosuppressive agents for the treatment of a variety of inflammatory and auto-immune conditions including organ transplantation, rheumatoid arthritis, lupus erythematosus, asthma, and allergic rhinitis. Our understanding of the critical endocrine, immune, and pharmacologic functioning of the body's response to MPL continuously improves as our ability to probe experimental models at diverse levels of functional organization (genomic, transcriptomic, proteomic and eventually metabolomic) also improves. In order to properly organize this wealth of -omics data and upgrade its information content, integrated computational analyses are required to unravel direct and indirect regulatory mechanisms of MPL. Our investigation, focusing on MPL response in the liver, kidney, and skeletal muscle tissue, aims to demonstrate a top-down, generalizable and expandable framework for augmenting dynamic pharmacokinetic and pharmacodynamic (PK/PD) models incorporating genomic, transcriptomic and proteomic information. We seek to extend our ability to describe steroid responses in ways of mechanistic, pharmacologic, and clinical relevance by developing complex models in the context of quantitative systems pharmacology (QSP). This investigation will help evolve quantitative pharmacologic models towards system-level integration providing insights into, and prediction of, the tissue- and dosage-dependent response to MPL.

This investigation is divided into multiple aims:

(Specific Aim 1 - completed) In aim 1, we developed a pathway-based analysis framework which analyzes temporal expression data and gleans global activities from pathways, returning information describing significantly populated and active pathways, as well as ranked lists of genes matched to these activities. This framework is designed to perform a previous knowledge based pathway analysis on expression data, simultaneously providing GSEA and expression activity information about a dataset.
(Specific Aim 2 – completed) In aim 2, we endeavor to characterize the consequences of acute MPL dosing within the liver. Characterization of these dynamics relies on the framework developed in Aim 1, with which response data to acute and chronic MPL dosing, from the liver will be analyzed. Through this work, we expect to deliver: 1) a better understanding of tissue/organ level effects of MPL administration using our combined PK, transcriptomic, and proteomic modeling approach described above, and 2) a modeling framework for examining the effects of other drugs and/or environmental factors.
(Specific Aim 3 – in progress) In aim 3, we expand our understanding of the tissue-specific response to MPL by characterizing response to MPL within the kidney and skeletal muscle tissue as well as characterizing baseline circadian pathway activity across multiple tissues from intact and drug free mammals. Characterization of these dynamics also relies on the framework developed in Aim 1, which will be applied to transcriptomic expression data harvested from ADX rat kidney and skeletal muscle tissue, post acute and chronic MPL dosing. The baseline circadian pathway activity analysis for intact drug-free rats seeks to characterize circadian pathway activity across multiple tissues (liver, adipose, skeletal muscle, and lung) in order understand the interaction of target tissue behavior and behavior due to administered corticosteroids.

PRESENTATIONS

Acevedo A, Berthel A, DuBois D, Almon RR, Jusko WJ, Androulakis IP. Pathway-Based Analysis of the Liver Response to Intravenous Methylprednisolone (MPL) Administration in Rats: Acute Versus Chronic Dosing. AIChE 2018, Pittsburgh, Pennsylvania, Oct 31, 2018.

Acevedo A, DuBois D, Almon RR, Jusko WJ, Androulakis IP. Global, Dynamic, Liver-Specific Transcriptomic and Proteomic Pathway-Based Analysis of In Vivo Responses Following Intravenous Methylprednisolone (MPL) in Rats. AAPS 2017, San Diego, California, Nov 15, 2017.

Acevedo A, Androulakis IP. Allostatic Breakdown of Multiple Homeostat Systems: A Computational Approach. BMES 2016 Annual Meeting, Minneapolis Minnesota, Oct 6, 2016.

Acevedo A, Androulakis IP, Bae SA. Asymmetry in Signal Oscillations Contributes to Efficiency of Periodic Systems. AIChE Annual Meeting in Salt Lake City, UT, Nov. 9, 2015.

AWARDS

GAANN, Department of Biomedical Engineering 2017 – Present

JEREMY ANDERSON
Advisor: Li Cai

Traumatic brain injury (TBI), defined as a mild to severe shock to the head that disrupts normal brain function, can result from sport injuries, vehicular accidents, and falls. TBI, which can lead to temporary or permanent loss of memory and motor function, was responsible for 2.2 million emergency department visits and 50,000 deaths in 2014 (CDC, 2015). The primary injury is irreversible, with treatments focusing on decreasing the secondary injury to minimize cell death and nervous tissue damage, which are often insufficient in patients with significant injury. However, TBI induces endogenous neural stem cell (NSC) activation, where the activated NSCs can integrate into neuronal circuitry and play a role in learning, memory, and motor functions. Unfortunately, the extent of NSC activation (e.g., proliferation, migration, differentiation) and the genes driving this NSC activation upon TBI are not well characterized. Understanding this NSC activation after injury will aid in the development of novel therapeutics promoting neurogenesis and functional recovery. Our overall objective is to investigate what cellular and transcriptome changes are induced in NSCs after TBI with hopes that the neurogenic response can be promoted to aid injury repair post-TBI. We believe that the endogenous NSCs respond to TBI and have the potential to recover TBI-induced cell damage. Understanding this response can identify genes associated with neurogenesis post-TBI and provide a basis for the development of new therapies. Our goal is to characterize the activation of endogenous NSCs after TBI to determine their potential in injury repair and neural regeneration by: 1) using a clinically relevant closed head injury (CHI) model with Notch1CR2-GFP transgenic mice, to identify GFP+ NSC response to injury, 2) determine the effect of Gsx1 in Notch1 signaling and neurogenesis after TBI.

For the first aim, we characterized the neurogenic response in CHI-injured Notch1CR2-GFP transgenic mice where Notch1 activity drives a GFP reporter for visualization of NSCs. Transgenic mice 8-12 weeks of age were injured and GFP+ NSCs were quantified at 1, 2, 3, 5, and 7 day(s) post injury (dpi). Mice were

screened for GFP expression in the hippocampus, lateral ventricle, and olfactory bulb, known regions of higher NSC presence. Using immunohistochemistry, we identified that GFP+ NSCs are expressed in higher levels after injury compared to control/sham animals with a peak at 2 dpi, and confirmed that GFP+ NSCs express quiescent neural progenitor markers, active neural stem cell markers, and mature neuron markers (e.g., Nestin, DCX, NeuN).

The second aim is to define the role of Gsx1 expression on NSCs and injury-induced NSCs after TBI, through overexpression of Gsx1 in the brain by lentiviral delivery. We believe Gsx1 plays a role in NSC injury recovery due to 1) Gsx1 role in Notch1 pathway in NSCs and 2) preliminary studies in SCI demonstrating that Gsx1 overexpression increases NSCs and decreases glial scar formation. We have generated the plasmid/lentivial delivery vector and produced the virus to deliver *in vivo*. We are currently delivering the lentivirus into the brain in sham and TBI animals to evaluate the effect on cellular and molecular NSC response after brain injury.

Understanding the characteristic activation of NSCs after TBI and its associated Notch1 genes will help elucidate the changes induced by TBI, and to determine NSC potential in injury repair and neural regeneration which will ultimately provide a foundation for the development of future therapeutics.

PRESENTATIONS

Anderson J. Endogenous Neural Stem Cell Activation after Traumatic Brain Injury. Biotech Annual Symposium, Piscataway, NJ, June, 2018

Anderson J. Traumatic Brain Injury-Induced Endogenous Neural Stem Cell Activation. NEBEC Regional Conference, Philadelphia, PA, March 2018

Anderson J. Endogenous Neural Stem Cell Activation after Traumatic Brain Injury. BMES National Conference, Phoenix, AZ, October 2017

Anderson J. Endogenous Neural Stem Cell Activation for Traumatic Brain Injury Treatment. Biotech Annual Symposium, Piscataway, NJ, June, 2017

Anderson J. Activating Endogenous Neural Stem Cells for Traumatic Brain Injury Treatment. NEBEC Regional Conference, Newark, NJ, April 2017

Anderson J. Endogenous Neural Stem Cell Activation for Traumatic Brain Injury Treatment. JMBGSA Symposium, Piscataway, NJ, March 2017

Anderson J. Endogenous Neural Stem Cell Activation for Traumatic Brain Injury Treatment. Biotech Annual Symposium, Piscataway, NJ, June, 2016

AWARDS

School of Engineering Fellowship 2014-2015

ANDREW BORELAND
Advisor: Peng Jiang

The lack of available human brain tissue for research has impeded our progress in understanding human neurobiology. Recent developments in induced pluripotent stem (iPS) cell technology has provided us with the ability to generate human stem cell-derived cerebral organoids. 3D organoids more accurately recapitulate human brain development compared to a more artificial 2D culture. Therefore, this platform presents a unique opportunity for disease modeling and developmental studies. However, despite progress organoids are not mini-brains and are far from perfect. For instance, they currently lack an important non-neuronal cell type, microglia.

I am becoming proficient in developing stem cell-derived organoid cultures and using patch-clamp electrophysiology to assay functionality. I created two microglial lines, one human derived iPS cell line and one green fluorescent protein (GFP)-expressing human embryonic stem (hES) cell-derived line. These lines are a powerful and robust tool for probing microglial influence on synaptogenesis, synaptic refinement, and neural network activity. My current work presently has two aims: The first is to investigate the role of human stem (hS) cell-derived microglia in shaping neural development using a hS cell-derived 3D cerebral organoid system co-cultured with hS cell-derived microglia. The second aim is to interrogate *in vivo* species-specific differences of microglia by generating human-mouse chimeric organisms using immune-deficient mice.

Additionally, I am working on a joint-project to create an HIV organoid model. I will be infecting my stem-cell derived microglia with replication competent HIV, JR-FL and VBA strains, and then combining them with pre-neural progenitor cells to form a cerebral organoid. The preliminary goals are to conduct single-cell sequencing on these organoids and assess transcriptional differences between infected vs non-infected cultures. I also hope to study how HIV infection is spread in these organoids, since most *in vivo* transmission occurs from direct cell-to-cell contact and not free-floating virus.

ALEXANDRA BURR
Advisor: Biju Parekkadan

Many endocrine disorders and hormone imbalance-based disorders rely on pharmaceutical solutions to supplement deficiencies with synthetic hormones that require daily medications, taken at very specific times of day for efficacy. In patients with these diseases such as thyroid deficiency, Parkinson's disease, or diabetes to name a few, patient compliance and adherence is a major limitation in the effectiveness of the treatment. Additionally, the dynamic nature of hormonal balance in the endocrine system make pharmaceutical solutions ineffective at matching endogenous release profiles and maintaining concentrations within the therapeutic window. Many hormones are regulated in a diurnal manner, where blood plasma levels peak either during the day or night, and in many cases, such as with melatonin, can run parallel with circadian rhythms. Circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) and effector cells that contain "clock genes" The promoters of these genes contain response elements that control transcriptional activation of downstream genes, making "clock" promoters potential targets for hormone secretion regulators. We propose a gene therapy to introduce "clock"-driven hormone secretion and provide natural protein therapeutics that will better match endogenous release profiles. In the case of melatonin, a "clock" promoter that peaks during

circadian night will be used to drive the transcription of melatonin genes to provide eventual protein release in the blood stream. We are developing constructs packaged in adeno-associated virus (AAV) serotypes that target skeletal muscle to then be administered intra-muscularly. Transduced muscle cells will then secrete melatonin in an oscillatory manner.

My current work has focused on developing "clock" constructs in AAV that drive a secreted reporter molecule such as luciferase. *In vitro* transductions have been done to verify that oscillatory secretion is being achieved. Additionally, I am working to develop novel methods for increasing transduction efficiency and expression longevity in muscle cells. To use a similar cell type that will be injected in vivo, we will be using a murine myoblast line, C2C12 to study both premature and differentiated muscle cells. My goal for the semester is to produce constructs that successfully secrete therapeutic proteins driven by constitutive promoters for later use in "clock" constructs.

PATENTS

Burr A, Parekkadan B, Tamayo A. Personalized and Timed Release Of Therapeutic Biomolecules Through Gene Therapy. RU Docket #2019-027 submitted, provisional patent in process.

LARRY CHENG
Advisor: Bin Tian

The placenta is a vital organ in fetal development. It serves critical functions as the lung and kidney of the fetus as well as produces human chorionic gonadotropin (hCG) to maintain the pregnancy. The main cells in the placenta are the trophoblast cells, which are further delineated into subtypes. The syncytiotrophoblast produces hCG and forms a physical barrier between the mother and the fetus. The cytotrophoblasts form the germative layer and fuse together to for the syncytiotrophoblast. The extravillous trophoblasts invade the decidua to remodel the uterus and anchor the placenta. Disorders related to the placenta contribute to a wide range of pregnancy-related disorders, from miscarriages and stillbirths to pre-eclampsia and fetal growth restriction. Clinical challenges regarding diagnosis and management of placental abnormalities is limited by the current lack of understanding of the biology of trophoblasts, including gene regulatory mechanisms.

Alternative polyadenylation (APA) is an RNA-processing mechanism that generates distinct 3'termini. Selection of the polyadenylation site (PAS) is influenced by many factors and results in modification of gene expression. Cleavage and polyadenylation at a PAS in the coding region leads to different protein isoforms with altered function. APA in 3'-untranslated regions (3'UTR) lead to distinct mRNA isoforms with different 3'UTR lengths but translate into the same protein. Differences in 3'UTR lengths cause these mRNA isoforms to be uniquely regulated by miRNA and RNA-binding proteins that affect their metabolism in the cell.

More than half of human mRNA encoding genes have multiple PAS sites, making them amenable to post-transcriptional control by APA. Distinct APA patterns have been reported in different tissues and conditions. Contrary to observations in the differentiation of embryonic stem cells into other lineages, preliminary data suggests that 3'UTR lengths are globally shorter upon differentiation into the

trophectoderm lineage. Therefore, we hypothesize that APA uniquely regulates the development and function of trophoblasts. This project seeks to add to our limited understanding of post-transcriptional regulatory mechanisms in the placenta.

Specific Aim 1: Analyze deep sequencing data from *in vitro* trophoblast models.
Hypothesis: APA regulation in trophoblasts is conserved between humans and mice.
Due to logistical and ethical challenges with acquiring *in* vivo samples, we conducted 3' region extraction and deep sequencing (3'READS) for trophoblasts differentiated from human and mouse embryonic stem cells (hESC and mESC, respectively). In addition to the preliminary data on hESC-derived trophoblasts, we have confirmed that global 3'UTR lengths are also shorter in mESC-derived trophoblasts, indicating that this phenomenon may be conserved across mammals. We will also perform Gene Ontology analysis as well as Ingenuity Pathway Analysis to identify biological processes and gene pathways affected by APA regulation. Interesting findings of mRNA isoforms will be confirmed by qPCR.

Specific Aim 2: Assess the consequences of APA regulation in *in vitro* trophoblast models.
Hypothesis: Global changes in 3'UTR length in trophoblast differentiation influences RNA metabolism, affecting genes important to trophoblast function.
We have collected polysome profiling data of mESC-derived trophoblasts as well as JEG-3 and HTR-8/SVneo human trophoblast cell lines. The polysome, monosome, and free fractions of RNA are collected and sequenced by our 3'READS method. Global analysis of polysome profiling data will allow us to evaluate how APA impacts translational efficiency of mRNAs. We will determine how translational efficiency of genes changes upon differentiation into trophoblasts. Because the two cell lines exhibit different trophoblast features, these models may allow us to infer differences in RNA metabolism between trophoblast subtypes. Any key events in RNA isoform abundances will be validated by qPCR and key assertions made in protein production will be confirmed with western blots.

Specific Aim 3: Evaluate the physiological relevance of APA regulation in pregnancy.
Hypothesis: APA plays a role in the function of trophoblast subtypes in the human placenta.
We have identified a few RNA-seq datasets in the public domain that were derived from clinical specimens, including placentas at term and from early terminations. Preliminary analysis of one dataset suggests that syncytiotrophoblasts have widespread shortening of 3'UTR compared to cytotrophoblasts in two out of three placentas. Further confirmation of this observation will be assessed in a separate dataset. Furthermore, two public trophoblast datasets were generated by single-cell RNA-Seq. Unlike typical bulk RNA-Seq, single-cell RNA-Seq data will allow for greater granularity to assess APA patterns in the three trophoblast subtypes.

AWARDS

Presidential Fellowship, Rutgers University 2018
2nd Place, MBGSO Annual Symposium Poster Presentation 2018
The Martin L Yarmush Award for Outstanding Poster Presentation 2017
Proteomics Interdisciplinary Research Award, Institute of Quantitative Biomedicine 2016
Joint PhD Excellence Award, Institute of Quantitative Biomedicine 2016
Molecular Biosciences Excellence Fellowship, Rutgers University 2015

SKYLAR CHUANG
Advisor: Ki-Bum Lee

Atherosclerosis is a major contributor to coronary heart disease, which is the number one cause of death in the U.S. There are several causes of inflammation underlying atherosclerosis, such as cholesterol crystal formation, disturbed local blood flow, and hypoxia. In particular, cholesterol crystals have been found in atherosclerotic lesions and shown to induce inflammation by activating the NLRP3 inflammasome pathway. This leads to the secretion of downstream cytokines, such as interleukin-1β (IL-1β), which trigger an inflammation cascade. However, currently there is no clinically effective treatment for cholesterol crystals. In one recent study however, it was shown that beta cyclodextrin (β-CD) can degrade cholesterol crystals *in vivo* and promote plaque regression by enhancing cholesterol efflux in foam cells. Inspired by this discovery, we decided to construct a β-CD decorated, lipid bilayer-supported silica (LBS) nanoparticle that can act as a "cholesterol sponge" for targeted degradation and removal of cholesterol crystals.

My work so far has focused on the synthesis and characterization of the LBS nanoparticles. Successful synthesis was achieved using two silanizing agents, tetraethyloxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES) on the pre-synthesized liposomes. The TEOS acts as the main precursor of the silica matrix while APTES provides additional functional groups for grafting targeting antibodies and β-CD. Transmission electron microscopy showed that the LBS nanoparticles were approximately 100-150 nm, and were capable of binding to cholesterol crystals. I also performed filtration assays by incubating the LBS nanoparticles with fluorescent cholesterol crystals and found that these crystals became degraded versus the control condition. Interestingly, the silica shell that were etched more were able to degrade more cholesterol crystals compared to the silica that were less etched. This could suggest that more etching provides a greater surface area, allowing more β-CD to be grafted onto the surface of the LBS nanoparticles. To better understand how the LBS nanoparticles interact with cholesterol crystals inside cells, I performed cellular uptake experiments in human THP-1 derived macrophages. Preliminary fluorescence microscopy results showed that the LBS nanoparticles co-localized with the cholesterol crystals in cells. In the next few months, I am looking to perform further characterization studies (such as investigating the correlation between silica pore size and the amount of cholesterol crystals degraded) as well as cell viability assays and qPCR experiments looking at the inflammation pathway to better determine the efficacy of the LBS nanoparticles.

AWARDS

CSBLB CNSM Outstanding Thesis Award 2018
CSULB Graduate Dean's List of University Scholars and Artists 2017
McAbee-Overstreet Scholarship 2015
1st place, Physical Sciences, 29th Annual CSU Student Research Competition 2015
1st place, Physical Sciences, 2015 CSULB Student Research Competition 2015

MOLLIE DAVIS
Advisor: Martin Yarmush

The goal of my work is to develop a multi-modal approach to target osteoarthritis (OA). OA is the leading cause of disability in adults in the US. There are an estimated 1 in 3 people between the ages of 18-65 have some form of arthritis, and OA is the most prevalent type. OA is a disease of the entire joint, not just a simple degenerative wear and tear. As in all forms of arthritis, inflammation plays an important role in OA, however the role is largely regulated by chemokines and cytokines. These pro-inflammatory factors help drive the production of proteolytic enzymes that are responsible for degrading the ECM. Therefore, treatments for OA focus on three main components: pain, inflammation, and regeneration. To target pain and inflammation, NSAIDs and opioids are treatment options and are beneficial in the short term. However, side effects, including kidney and gastrointestinal issues, as well as their addictive nature, make them ineffective for long term pain management. Local anesthetics (LA), on the other hand, are generally non-toxic, but their duration is short. Therefore, a sustained release LA system would help prolong pain mitigation effects without the negative side effects associated with NSAIDs and opioids. Aside from pain, one of the key aspects of OA is inflammation. Mesenchymal stromal cells (MSC) are multipotent stem cells that can control multiple physiological functions via paracrine secretion. However, LAs can affect MSC viability and function. The Yarmush lab previously has shown that in the presence of LAs, MSC secretion and, therefore, anti-inflammatory function, is altered. Therefore, an improved method of co-administration of LA and MSCs is necessary.

We have developed a sustained release LA delivery model that could enable the co-administration of LA and MSC. Liposomes containing bupivacaine were encapsulated in an alginate matrix, which enables the sustained release of bupivacaine as compared to bupivacaine-containing liposomes alone. *In vitro* analysis indicated that using the construct compared to bolus bupivacaine increased MSC viability and cell secretion of prostaglandin E2 (PGE2), an anti-inflammatory cytokine, which indicates better anti-inflammatory properties and overall functionality.

Since the overall goal is to create a dual therapy, a method of stabilizing the cells at the injury site is necessary. Our lab has previously used alginate microspheres to encapsulate the cells to provide positional control. Moreover, our recent studies indicated that by encapsulating the MSC, the cells are protected from the LA. Therefore, higher doses of LA can be used in conjunction with eMSC in order to reach clinically relevant LA levels while still promoting eMSC viability and immunomodulatory secretion. These results indicate that the alginate encapsulation provides several therapeutic benefits including 1) limiting LA diffusion into the cell containing alginate microenvironment, and 2) positional control of MSC for a co-therapy with LA.

Current studies include analysis of the LA construct in the presence of other cell types involved in OA, such as chondrocytes and synoviocytes. This is important in order to create a more robust *in vitro* model of OA to be used with the dual therapy. In addition, I will be exploring the use of different types of nano- and micro-particles. While liposomes can promote sustained release of LA, a therapy for a chronic disease needs a longer lasting sustained release pain system than liposomes can deliver. Polymeric nanoparticles have been shown to improve release for up to 35 days. Current studies are ongoing in PLGA nano- and micro-particle formation as well as analyzing diffusion profiles of LA via HPLC. Furthermore, these nano- and micro-particles can be used to encapsulate additional factors to promote cartilage regeneration and inflammation, such as TGF-β. In addition, in order to understand how pain and inflammation interact in OA, the relationship between the two components will be studied to determine a more comprehensive understanding of OA as well as help determine more specific targets for OA therapies.

PRESENTATIONS

Davis M, Marrero-Berrios I, Perex XI, Rabolli C, Schloss R, and Yarmush ML. An Integrated Anesthetic-MSC Therapeutic. BMES Conference, Atlanta, GA, October 18 2018.

Davis M, Marrero-Berrios I, Perex XI, Rabolli C, Schloss R, and Yarmush ML. An Integrated Anesthetic-MSC Therapeutic. SACNAS Conference, San Antonio, TX, October 11 2018.

Davis M, Maguire T, Marrero-Berrios I, Zhu C, Gaughan C, Schloss R, and Yarmush ML. Control Release Anesthetics to Enable and Integrated Anesthetic-mesenchymal Stromal Cell Therapeutic**.** BMES Conference, Minneapolis, MN, October 6 2016.

AWARDS

US Department of Education GAANN-Match Fellowship 2015-2016

EMILY DI MARTINI
Advisor: David Shreiber

Targeted drug delivery is a promising approach to enhance the accumulation and prolong the presentation of therapies in diseased and injured tissues. Targeted systems reduce the dosage that is required, thereby limiting off-site and systemic effects. Ligand-receptor interactions are traditionally identified to selectively deliver therapies, and while this approach can have great specificity, it can be costly and suffer from limited sensitivity. Rather than relying on receptor-specific interactions, an emerging approach is to target intermediary species that modulate disease progression. We have recently demonstrated the use of native free radical concentrations as a homing signal in proof-of-concept studies. Elevated concentrations of free radicals are a characteristic co-morbidity of many different injury and disease conditions. In polymer chemistry, free radicals are frequently used to initiate crosslinking reactions. We propose that free radicals that are typically elevated in diseased and injured tissues are capable of inducing crosslinking of acrylate groups on polymer chains, such as polyethylene glycol diacrylate (PEGDA). Coupling payloads to the acrylated polymer would then allow for specific targeting and immobilization of these payloads to areas with elevated concentrations of free radicals. In vitro, we have demonstrated that reactive oxygen species (ROS) can initiate crosslinking of acrylated PEGs, which enables the immobilization of a fluorescent payload within tissue mimics. The crosslinking efficiency and immobilization potential varied with the polymer chain length, which suggests that a tunable platform can be achieved.

In my first year of graduate school, my time was primarily devoted to coursework. In the lab, I assisted in characterizing the immobilization and reaction kinetics of our acrylated polymers. Additionally, we demonstrated that the reaction of these functionalized PEGs with free radicals protected cells from the damaging effects of oxidative stress in vitro. As I transition to my second year, I have focused on investigating functional outcomes of a therapeutic payload. We successfully grafted PEG acrylates to platelet derived growth factor (PDGF) and confirmed that the growth factor retained bioactivity. We plan to immobilize PDGF in a tissue mimic and quantify the proliferation of cultured fibroblasts. Moving forward, our preliminary results indicated that, in addition to acrylates, alternate functional chemistries

such as thiols and alkenes also react with free radicals for immobilization. Additionally, we found that a cocktail of molecular weight PEGs – short chains to drive formation of a network and large chains to promote immobilization of the network – may enhance the functional delivery of our therapeutic payload. We also believe transitioning to a two-component, two-phase "clickable" catch-and-release system will provide significant improvements in forming the polymer network and allow for temporal and tune dosing. Ultimately, this improved platform has the potential to serve as a building block for improved administration of many therapeutic payloads to patients suffering from a variety of ailments.

PRESENTATIONS

DiMartini E, Lowe C, Mirmajlesi K, Gormley A, Shreiber DI. Targeted Delivery of Therapeutic Factors via Free Radical Mediated Immobilization. 2018 BMES Annual Meeting, Atlanta, GA.

DiMartini E, Lowe C, Mirmajlesi K, Shreiber DI. Free-radical scavenging potential of acrylated polyethylene glycol polymers for TBI treatment. 2016 BMES Annual Meeting, Minneapolis, MN.

PATENTS

Lowe C, **DiMartini E**, Gormley A, Shreiber D. In Vivo Radical-Mediated Polymerization for Targeted Delivery of Trophic Factors. U.S. NonProvisional Patent Application No. 16/009,033, filed June 14, 2018

ZACH FRITZ
Advisor: Martin Yarmush

Cancer is the second leading cause of death in the US, but early detection through screening methods can lead to lower mortality rates and more effective treatments. While imaging modalities are commonly used to screen for cancer, these can be expensive, potentially expose a patient to ionizing radiation, and may not be able to distinguish between benign and malignant tumors. It has been found that many malignancies can induce the production of autoantibodies via mutation and/or over-expression of tumor associated antigens. These autoantibodies, such as those specific to the tumor suppressor protein p53, can be detected in at-risk patients, years before formal cancer diagnosis, making them potentially powerful disease biomarkers. However, current immunoassays for their detection suffer from low sensitivities; for example, even in cancers with the highest frequency of p53 mutation, autoantibodies are only detected in at most 35% of patients. While it is undoubtedly true that not all cancer patients will exhibit these autoantibodies, we believe that the choice of capture antigens used in an immunoassay is critical to its efficacy, and that current assays are using antigens (whether whole proteins or truncated peptides) that might not be representative of a patient's polyclonal autoantibody response.

For this project, we have developed a novel, coarse-grained bioinformatics model that can translate sequence data from a protein or peptide into information about that protein's energy profile. We hypothesize that this model could be used to increase the sensitivity of autoantibody immunoassays by aiding in the selection and design of antigenic peptides with a higher affinity for polyclonal autoantibodies. This includes the identification of novel antigens not previously considered for use in assays. Our long-term goals are to develop and apply this bioinformatics model for use in biomarker discovery and multipanel assay optimization for the detection of diseases like cancer. Our short-term

goals focus on proof of concept studies targeting a single analyte and demonstrating the model's potential for integration into an assay platform.

Specific Aim 1: Validate the protein energetics model's antigen optimization capabilities. The tumor suppressor protein p53 was chosen as our target antigen due to the ubiquitous presence of its mutations and autoantibodies in most solid tumors. Immunoassays will be carried out in proof-of-concept experiments using variations on a single p53 antigenic peptide designed using our model. The groups tested will include an unmodified control peptide, one optimized by the model for superior affinity, one predicted to have lower affinity than the control, and one predicted to have a higher degree of nonspecific binding. The control peptide will be modified by the addition of select flanking residues, which will influence the free energy of their neighboring residues and allow us to design peptides that more closely reflect the energetics of their whole protein counterparts. All antigens will be conjugated to microbeads in a multi-antigen, high loading format and tested against the same monoclonal anti-p53 antibody.

After validation and antigen refinement using monoclonal standards, we will use commercial serum samples that have been previously quantified for p53 autoantibodies as well as clinical cancer patient serum samples to further assess the assay's sensitivity and specificity when exposed to a typical polyclonal response. These results will be compared with those of our collaborators and to results obtained using commercially available p53 autoantibody ELISA kits.

Specific Aim 2: Develop a microfluidic assay platform with integrated detection capability. A microfluidic device offers an attractive assay platform because of decreased sample and reagent consumption and the potential for parallelization. Our specifications for the device include a module that facilitates efficient mixing of the antigen microbeads and antibody-containing samples, as well as integrated detection capabilities so that assay analysis can be done on-chip. The device's detection capability and sensitivity will be tested and compared to a well plate assay analyzed with a conventional benchtop microbead flow cytometer.

Specific Aim 3: Conduct a clinical study with cancer patient sera samples and healthy controls to assess the assay's ability to screen for cancer: Using sera samples from colorectal cancer patients and cancer-negative control samples obtained from our collaborators (including Robert Wood Johnson University hospital) and a biorepository (Cancer Institute of New Jersey), we will run the immunoassay on our device using a set of model-optimized peptide antigens derived from various parts of the p53 protein. We believe that our optimized immunoassay will outperform similar assays in terms of sensitivity (increased true positive rate), without sacrificing specificity (minimal effect on true negative rate).

To date, I have used the bioinformatics model to obtain the energetic profile of the p53 protein and have designed variants of a single peptide epitope with varying degrees of model-predicted affinity for antibodies. Preliminary immunoassay and surface plasmon resonance experiments with these peptides have supported the model, and further experiments with an expanded pool of peptides are planned. Testing and optimizing various components of the device (valves, mixing, membrane) has begun. Other milestones include publishing/submitting two papers, gaining IRB approval for the project, and establishing several collaborations.

PRESENTATIONS

Fritz Z, Yarmush ML, Schloss R, Shrirao A, Williams L. Improving Cancer Diagnostics with Protein Energetics Modeling and Microfluidics. BMES Annual Meeting, Atlanta, GA, October 17-20

Fritz Z, Yarmush ML, Schloss R, Shrirao A, Williams L. Improving Cancer Diagnostics with Protein Energetics Modeling and Microfluidics. Abcam Biomarkers in Immuno-Oncology Meeting, Boston, MA, March 1, 2018.

Fritz Z, Yarmush ML, Williams L. Using a Novel Protein Energetics Model to Improve Cancer Diagnostics. SAPA Oncology Symposium, Rutgers University, Piscataway, NJ, April 8, 2017

Fritz Z, Yarmush ML, Schloss R, Williams L. Using a Novel Protein Energetics Model to Improve Cancer Diagnostics. BMES Annual Meeting, Phoenix, AZ, October 11-13, 2017

MADISON GODESKY
Advisor: David Shreiber

Extracellular matrix (ECM) is an intricate network of bioactive molecules that maintains the basic architectural features of native tissues through the physical support and cell-signaling cues it provides. At the tissue-level, specific presentations of ECM mechanical and bioactive properties assist to regulate adhesive interactions between cells and the matrix. Through these anchor points, cells generate traction forces which act on the cytoskeleton, influencing gene expression and the matrix, providing feedback across various time and length scales. In native ECM as well as on synthetic scaffolds, mechanotransduction influences cell behaviors including adhesion, migration, and stem cell differentiation. Recently, bioprinting has gained attention as a high-throughput approach to design live-tissue replacements for basic research, rapid screening, pre-clinical testing, and regenerative medicine. Despite rapid advances in 3D printer technology, progress in bioprinting has remained limited by the availability of biomaterial “inks” that can be patterned to properly replace the important signaling context that native matrix provides. Our research suggests that a new bioink based on thiol-modified hyaluronic acid (HA-S) and polyethylene glycol diacrylate (PEGDA) may potentially fulfill this unmet need. We hypothesize that the dual crosslinking mechanism in HAS-PEGDA can be leveraged to independently tune the mechanical and bioactive features across multiple time and length scales. To test this hypothesis and develop a bioink system based on HA-S, we are pursuing the following three specific aims:

1. Establish a system to independently tune HAS-PEGDA mechanical and bioactive features: Extensive rheological testing confirms the crosslinking properties of HAS-PEGDA are dictated by two cytocompatible reactions and demonstrate that both the rate and extent of each reaction can be controlled. Like HA, which serves as a key mediator of cell attachment in native ECM, HAS-PEGDA is non-cell-adhesive without further modification. With these principles in mind, we developed a simple template to decouple and tune the mechanical and bioactive features at multiple time scales. By selectively targeting bioactives to the sites that otherwise form latent crosslinks, we determined the equilibrium mechanical properties can be tuned independently of the initial gelation reaction.

Additionally, by altering the ratio of bioactive ligands to inactive control sequences, we found that the bioactive properties can be decoupled from the equilibrium stiffness. In the presence of dermal fibroblasts and human mesenchymal stem cells (hMSCs), our results indicate that the range of mechanical and bioactive features on HAS-PEGDA can be tuned to significantly influence cellular adhesion, spreading, and migration on the gels.

2. Modify the system to be amenable for the encapsulation and infiltration of cells: Previous research with a variety of different cell types has consistently demonstrated that cells encapsulated inside HAS-PEGDA hydrogels survive, but do not spread or migrate in 3D. As a bioink for 3D printing, it was critical to modify the system to allow for cell encapsulation. To tune the porosity of HA-S while maintaining the range to control gelation dynamics and initial stiffness, we incorporated matrix metalloproteinase (MMP) degradable peptide sequences into the crosslinker backbone. We demonstrated that hMSCs encapsulated in MMP-2-degradable hydrogels can spread and migrate in 3D, while cells inside control gels crosslinked with a non-degradable peptide sequence remained rounded.

3. Pattern the biofunctional properties and demonstrate spatial guidance of cellular behavior: Previously, we have characterized the viscosity and gelation dynamics of HAS-PEGDA and demonstrated that the material can be patterned with extrusion-based 3D printing. Currently, we are developing a bioink-dispensing and mixing system and are validating that the composition of the bioink can be automated and synchronized to the movements of the 3D printer head. In parallel, we are building an *in vitro* model of dermal tissue to use as the proof-of-concept for this thesis. We aim to use the HAS-based bioink system to 3D print an *in vitro* model of the dermis that patterns the specialized structure of the hair follicle, which houses most of the stem cells and regenerative potential of the skin.

At the conclusion, we expect to have developed a versatile bioink system based on HAS-PEGDA. Like traditional printer inks which rely on four pigments – cyan, magenta, yellow, and black – to generate the entire color spectrum, we expect that bioinks based on HA-S, crosslinkers, and bioactives can be combined to produce a spectrum of matrix signaling features. From an engineering perspective, biomaterials such as HAS-PEGDA that offer independently tunable mechanical and bioactive features are advantageous for patterning specific matrix signaling cues, while limiting the non-specific cellular interactions with the device. HA-S is a new bioink material that offers a convenient template to pattern functionally-complex scaffolds and potentially replace the important signaling context that native matrix provides.

PRESENTATIONS

Godesky MD, Shreiber DI. Hyaluronic Acid-Based Bioinks for Cell-Friendly 3D Printing. 2018 Biomedical Engineering Society Annual Meeting, Atlanta, GA, October 18, 2018. (Accepted)

Godesky MD, Shreiber DI. A Hyaluronic Acid-Based Bioink for Blank-Slate Extracellular Matrix Design. Innovations in Dermatological Sciences: Future of Dermatologicals and Cosmeceuticals Conference, Center for Dermal Research, Woodbridge, NJ, October 8-9, 2018.

Godesky MD, Shreiber DI. Hyaluronic Acid-Based Hydrogels with Independently Tunable Mechanical and Bioactive Properties. 2017 Biomedical Engineering Society Annual Meeting, Phoenix, AZ, Oct 12, 2017.

Warren R, Kemraj A, **Godesky MD**, Shreiber DI, Baum J. The Impact of Vascular Ehlers-Danlos Syndrome Mutations on Integrin-to-Collagen III Binding. 2017 Biomedical Engineering Society Annual Meeting, Phoenix, AZ, October 12, 2017.

Godesky MD, Shreiber DI. Hyaluronic Acid-Based Hydrogels with Independently Tunable Mechanical and Bioactive Properties. Biotechnology Training Program Annual Symposium, Piscataway, NJ, June 8, 2017.

Godesky MD, Shreiber DI. Hyaluronic Acid-Based Hydrogels with Simultaneously Tunable Mechanical and Bioactive Properties. 2016 Biomedical Engineering Society Annual Meeting, Minneapolis, MN, Oct 6, 2016.

Godesky MD, Basarkar A, Sharma M. Syringe Compatibility of High-Concentration MK-3475. Merck Research Laboratories, Biologics Technical Review Meeting, Kenilworth, NJ, July 18, 2016

Godesky MD, Shreiber DI. Hyaluronic Acid-Based Hydrogels with Simultaneously Tunable Mechanical and Bioactive Properties. Biotechnology Training Program Annual Symposium, Piscataway, NJ, June 7, 2016.

Lebron A, **Godesky MD**, Shreiber DI. Co-modulating the Mechanical and Bioactive Properties of CMHAS-PEGDA Hydrogels for Neural Tissue Engineering. Biomedical Engineering Society 2015 Conference, Tampa, FL, October 7-10, 2015.

Godesky MD, Shreiber DI. Rheological Characterization of Hyaluronic Acid-Based Hydrogels for Peripheral Nerve Repair. Biotechnology Training Program Annual Symposium, Piscataway, NJ, June 24, 2015.

AWARDS

The Alliance for Regenerative Rehabilitation Research Tech Development Grant (In Progress)
GAANN Fellowship in Precision & Personalized Medicine Fellowship 2018-19
Graduate Research Fellowship (NIH-NINDS1R01NS078385) 2016-17
U.S. Department of Education GAANN Fellow 2014-15

RYAN GUASP
Advisor: Monica Driscoll

Advanced age is a major risk factor for neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. These diseases are increasing in prevalence as the population of older Americans continues to expand. One pathological hallmark shared by these three diseases is the formation of protein aggregates in the brain. Using the nematode Caenorhabditis elegans as a model organism, the Driscoll lab has recapitulated the aggregating protein phenotype by expressing human huntingtin protein, or multiple copies of mCherry fluorescent protein in its mechanosensory neurons. A surprising discovery was made of a heretofore unreported ability of the neurons to jettison these protein aggregates, alongside damaged mitochondria and large quantities of cytoplasm in a single, massive (average 3.8-µm diameter) extracellular vesicle, which we term the exopher. Although producing an exopher is a rare neuronal event (approximately 7% chance in strains expressing aggregating proteins), we have shown that releasing one correlates to improved cellular function later in life.

My first specific aim is to elucidate the exopher mechanism by genetically characterizing the cellular machinery responsible for selecting, transporting, and ejecting exopher cargo. I have previously performed genetic screens using RNA interference (RNAi) to knock down expression of genes which I hypothesized might be relevant to exopher-genesis. I identified several genes that can inhibit exopher formation including genes that encode molecular motors, cytoskeletal proteins, and polarity proteins, as well as two potent inhibitors with no previously known adult functions. Additionally, I found that genes required for the production and release of well-characterized extracellular vesicles seem to play no role in exopher-genesis. Extending on this work, I have used gene network analysis software (Cytoscape 3) to map previous hits and all known interacting genes as recorded on WormBase, the C. elegans genomics database. I am using RNAi to investigate each gene in the network linking at least two other nodes in an iterative process. This process has revealed a number of genes which suppress exopher formation that are primarily known to function in the C. elegans germline. This comports with findings in the lab that chemical disruption of C. elegans fertility can abrogate exophers production. I am pursuing this finding to look for a transcellular signal from the gonad that may control the timing of exophers production.

My second specific aim is to characterize the ultrastructure of the exopher and its cargo at the sub-organellar level. Using high-pressure freeze fixation and transmission electron microscopy the lab has produced the first EM images containing exophers. An exopher produced by an ALMR mechanosensory neuron is completely surrounded by the multinucleate hypodermal syncytium, hyp 7. It is multi-membrane bound with at least 4, and as many as 20, membrane layers surrounding various sub-compartments. Whether the membranes originate from the neuron, or from the surrounding hypodermis remains to be elucidated. The exopher displays a globular morphology and is divided into compartments that contain distinct cargo; membrane whorls and vacuoles are discernible, along with dense inclusions that may be the mCherry aggregates we observe with fluorescence microscopy. We are working on processing additional samples to try to capture the distinctive filament that attaches some exophers to the originating neuronal soma. An EM image of an exopher definitively containing a mitochondrion is a priority because evidence in the literature shows that mitochondria can be ejected from murine retinal ganglion neurons and superficial cortical layers and degraded in neighboring astrocytes.[1] If exophers are evolutionarily conserved in mammals, this research may offer insights into mechanisms of neurodegenerative pathology, as well as targets for novel therapeutic agents in several diseases. Several hundred samples have now been fixed and are under analysis.

My third aim is to develop a system that allows exophers to be studied in a more physiologically normal context. Anecdotally in the lab, we have noticed exophers forming and moving away from the soma more quickly when viewed in Petri dishes under low magnification fluorescence dissecting microscopes, than when mounted between a slide and coverslip. When grown on an agarose plate, C. elegans is perpetually initiating sinusoidal movements and pharyngeal pumping to continuously eat bacteria. Worms undergo a metabolic shift to a starvation state after minutes without food, and under our current time lapse microscopy protocol, an exopher can take up to an hour to form. To screen for exophers under a higher-power fluorescence microscope, C. elegans is mounted on a slide and paralyzed using the anthelmintic drug, tetramisole, in a buffer without bacteria. I aim to create a microfluidic device that will allow exopher-genesis to be studied at high magnification, while the worms are able to freely move in individual chambers that are continuously perfused with bacteria. To investigate a potential mechanical component to exopher-genesis or whether it is metabolically-influenced, we need conditions where C. elegans move and feed freely.

1. Davis CH, et al. Transcellular degradation of axonal mitochondria. Proceedings of the National Academy of Sciences of the United States of America 111, 9633-9638, doi:10.1073/pnas.1404651111 (2014).

PRESENTATIONS

Guasp R, Melentijevic I, Arnold M, Harinath G, Driscoll M. Genetic dissection of mechanisms that contribute to extrusion of neurotoxic aggregates from neurons. 20th International C. elegans Conference, University of California, Los Angeles, June 24 - June 28, 2015

Melentijevic I, Toth M, Arnold M, **Guasp R**, Harinath G, Parker A, Neri C, Driscoll M. Neuronal Exophers: a novel mechanism for the removal of neurotoxic cytoplasm components. 20th International C. elegans Conference, University of California, Los Angeles, June 24 - June 28, 2015

Toth M, Ganihong I, Gaul K, Patel K, **Guasp R**, Harinath G, Zhang W, Xue J, Driscoll M. Novel gene interactions that modulate morphological aging of neurons in C. elegans. 20th International C. elegans Conference, University of California, Los Angeles, June 24 - June 28, 2015

Smart J, Melentijevic I, Harinath G, Toth M, **Guasp R**, Arnold M, Driscoll M. Touch neurons can toss out mitochondria. 21st International C. elegans Conference, University of California, Los Angeles, June 21 - June 25, 2017

Guasp R, Arnold M, Melentijevic I, Harinath G, Toth M, Nyguen K, Taub D, Gabel C, Xue J, Hall D, Driscoll M. Structural Components and Genetic Requirements of Exophers and their Formation. 21st International C. elegans Conference, University of California, Los Angeles, June 21 - June 25, 2017

Abbott M, Melentijevic I, **Guasp R**, Driscoll M. A novel high-throughput whole-genome RNAi screening technology utilized to investigate the molecular pathways of exopher production. 21st International C. elegans Conference, University of California, Los Angeles, June 21 - June 25, 2017

Arnold M, **Guasp R**, Melentijevic I, Grant B, Driscoll M. Trash collection before exopher ejection. C. elegans SPAMS meeting, University of Madison, Wisconsin, June 28 - July 1, 2018

Chia WK, **Guasp R,** Arnold M, Driscoll M. Probing the relationship between exophers and lysosomes. C. elegans SPAMS meeting, University of Madison, Wisconsin, June 28 - July 1, 2018

Guasp R, Thawani C, Salam S, Driscoll M. Gonad-induced proteostatic remodeling at the onset of adulthood may influence exopher production. C. elegans SPAMS meeting, University of Madison, Wisconsin, June 28 - July 1, 2018

Smart J, Melentijevic I, Harinath G, Toth M, **Guasp R,** Arnold M, Driscoll M. Touch and dopaminergic neurons eject mitochondria under native stress conditions. C. elegans SPAMS meeting, University of Madison, Wisconsin, June 28 - July 1, 2018

AWARDS

AAAS/Science Program for Excellence in Science May 2016
Poster in Neurobiology Honorable Mention, 21st International C. elegans Conference June 2017

PAULINA KRZYSZCZYK
Advisor: Francois Berthiaume

Chronic wounds affect millions of Americans. These wounds are non-healing and reach deep into the skin. On the tissue level, they are characterized by a persistent inflammatory state and hypoxia. A main reason as to why chronic wounds are stalled in inflammation is due to the presence of M1 macrophages, which produce high levels of pro-inflammatory cytokines and damaging reactive oxygen species (ROS). Under normal wound healing, M1 macrophages transition to anti-inflammatory M2 macrophages as tissue regenerates, but this does not occur in chronic wounds. We are investigating the use of hemoglobin (Hb) as a chronic wound therapy mainly due to its potential to promote the M2 macrophage phenotype via the heme-oxygenase 1 (HO-1) pathway. Hb can also be used as an oxygen delivery vehicle to hypoxic wounds. We take two approaches to utilizing Hb to elicit beneficial wound healing effects: 1) by polymerizing hemoglobin (forming PolyHb) and 2) in combination with haptoglobin (Hp).

The rationale behind the use of PolyHb is that oxygen delivery can be tuned by polymerization under fully oxygenated or deoxygenated conditions (relaxed/R-state and tense/T-state, respectively). Additionally, PolyHb reduces protein unfolding and disassembly, thereby protecting against the release of the toxic free heme group. PolyHb is acquired in collaboration with Dr. Andre Palmer's lab at The Ohio State University. We have investigated the effects of Hb/PolyHb on ROS generation, cell shape and cell attachment through *in vitro* studies with differentiated human M1 macrophages from three different donors. We found that T-state PolyHb consistently reduced ROS generation whereas Hb elongated cells and promoted attachment. We have also observed that macrophages treated with higher concentrations of Hb for 48 hours have a lower attached cell number compared to incubation PolyHbs. There is also higher interleukin 10 (IL-10) and lower tumor necrosis factor-α (TNF-α) production. This suggests that Hb is promoting anti-inflammatory effects, whereas PolyHb is not, possibly due to the difference in ability to activate the HO-1 pathway. There is also ongoing analysis of a larger panel of 27 inflammation-related proteins produced from macrophages treated with Hb/PolyHbs.

The rationale behind the use of Hp in conjunction with Hb is that these proteins bind tightly to each other and to CD163 receptors on macrophages. The Hb:Hp complex is endocytosed, thereby activating the anti-inflammatory HO-1 pathway, which has shown benefits in promoting wound healing. To study the effect of Hb:Hp complexes on a wide array of secreted proteins, multi-plex immunoassays were performed. The secretion of 27 inflammation-related cytokines, chemokines and growth factors were measured from the supernatants of M1 macrophages cultured in media, Hp alone, Hb alone or Hb:Hp complexes. Some factors, such as IL-8 and IL-17, were significantly decreased below baseline following Hb:Hp treatment. In other factors (*e.g.* interferon-γ and TNF-α), Hb alone significantly increased secretion above baseline, whereas Hb:Hp complexes brought levels back down. Similar studies were performed on M2 macrophages as well, since wounds contain a heterogeneous population of macrophages. Current efforts are being made to extract biologically-relevant information and clusters of trends from these datasets using Ingenuity Pathway Analysis and Principal Component Analysis.

The next step for this project is to test the effect of these cytokine-containing supernatants in an *in vitro* wound model (scratch assay) and measure the rate of closure of cultured fibroblasts, keratinocytes and endothelial cells. These results will yield insight on which treatment and resulting cytokine cocktail is most beneficial for wound healing. This work focuses on decreasing inflammation, which is a key barrier to wound healing. By promoting an anti-inflammatory phenotype in macrophages, skin cells will be primed for repair.

PRESENTATIONS

Krzyszczyk P, Olabisi R. Growth Factor Release from Mesenchymal Stem Cells Encapsulated in PEGDA Hydrogels. 2014 BMES Annual Meeting, San Antonio, TX, October 24, 2014.

Krzyszczyk P, Faulknor R, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Polymerized Hemoglobin Accelerates Wound Healing in Diabetic Mice. 2015 BMES Annual Meeting, Tampa, FL, October 8, 2015.

Krzyszczyk P, Faulknor R, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Polymerized Hemoglobin Accelerates Wound Healing in Diabetic Mice. 2015 Innovations in Dermatological Sciences Conference, New Brunswick, NJ, November 10, 2015.

Krzyszczyk P, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Development of a Hemoglobin-Based Treatment to Promote M2 Macrophage Polarization in Inflammation. 2016 Innovations in Dermatological Sciences Conference, Iselin, NJ, September 29, 2016.

Krzyszczyk P, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Development of a Hemoglobin-Based Treatment to Promote M2 Macrophage Polarization in Inflammation. 2016 BMES Annual Meeting, Minneapolis, MN, October 7, 2016.

Krzyszczyk P, Patel K, Richardson K, Schloss R, Yarmush ML, Palmer A, Berthiaume F. In Vitro Macrophage Response to Hemoglobin-Based Treatments for Chronic Wounds. 2017 Innovations in Dermatological Sciences Conference, Iselin, NJ, October 2-3, 2017.

Krzyszczyk P, Patel K, Richardson K, Schloss R, Yarmush ML, Palmer A, Berthiaume F. Macrophage Response to Hemoglobin-Based Treatments for Chronic Wounds. 2017 STEM Community Outreach Symposium at Rutgers, Piscataway NJ, November 10, 2017.

Krzyszczyk P, Patel K, Richardson K, Schloss R, Yarmush ML, Palmer A, Berthiaume F. Effects of Polymerized Hemoglobin on Macrophage Response. 2017 BMES Annual Meeting, Phoenix, AZ, October 12, 2017.

Krzyszczyk P, Patel K, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Hemoglobin-Based Treatments to Promote Healing in Chronic Wounds. 2018 Johnson & Johnson Engineering Showcase, New Brunswick, NJ, February 21, 2018.

Krzyszczyk P, Patel K, O'Reggio M, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Secretome Analysis of Macrophages Treated with Hemoglobin-Haptoglobin Complexes. 2018 Innovations in Dermatological Sciences Conference, Iselin, NJ, October 9, 2018.

Krzyszczyk P, Patel K, O'Reggio M, Richardson K, Yarmush ML, Palmer A, Berthiaume F. Secretome Analysis of Macrophages Treated with Hemoglobin-Haptoglobin Complexes. 2018 BMES Annual Meeting, Atlanta, GA, October 18, 2018.

AWARDS

3 Minute Thesis Finalist (**top 33%** of participants) 2018
Johnson & Johnson Engineering Showcase Finalist (**top 20%** of applicants) 2018
Executive Women of New Jersey Graduate Merit Award 2017
1st Place Poster STEM Community Outreach Symposium at Rutgers 2017
2nd Place Poster Award, Innovations in Dermatological Sciences Conference 2015
US Department of Education GAANN Fellow 2015-2018

JOSH LEIPHEIMER
Advisor: Martin Yarmush

Venipuncture, the process of obtaining intravenous access for blood sampling or fluid delivery, is one of the most common clinical procedure performed worldwide, and also one of the most problematic. Because of accidental needle stick injuries and unnecessary long procedure times, difficult venipuncture costs the U.S. health care system on average 4.7 billion dollars every year. Previously in our lab, a benchtop robotic device was created to automate the venipuncture process. This device utilized 9 individual motors, a near-infrared (NIR) imaging system, and an ultrasound imaging probe to automatically identify a vein for cannulation and perform the needle insertion. However, its large size, lack of mobility, and high costs makes it impractical for clinical translation, as most venipuncture procedures will be done on patients that are either bed ridden or incapable of accessing the device. To solve the major limitations with the previous device, I plan to develop a hand-held, cost-effective, portable robotic venipuncture device, capable of both identifying a suitable vein for insertion, while also quickly, safely, and efficiently cannulating said target vein for difficult venous access patients. The device will utilize out-of-plane ultrasound imaging to segment suitable veins for insertion and utilize a custom force sensor feedback system for vein puncture detection. The device will be intended for easy clinical translation, featuring a simple to use interface that requires little to no human intervention or training required. By developing a hand-held, miniature device to quickly and safely automate venipuncture procedures, we plan to reduce injuries and problems associated with difficult venous access, while also reducing procedure times for healthcare clinicians, reducing healthcare related costs.

PRESENTATIONS

Leipheimer J, Balter M, Chen A, Maguire T, Yarmush ML. Investigating the Use of Structured Light Imaging for 3-D Reconstruction of the Human Forearm for Automated Venipuncture. Northeast Bioengineering Conference (NEBEC), Newark, NJ, March 31, 2017.

Leipheimer J, Balter M, Chen A, Maguire T, Yarmush ML. A Robotic Device for Automated Venipuncture. NJ Tech Council – What's Next in Medical Devices, Princeton, NJ, June 13, 2017.

Leipheimer J. Robotic Device for Automated Venipuncture – Eliminating Needle Stick Injuries. Rutgers Three Minute Thesis (3MT) Competition, Cook Campus, Rutgers, March 2018.

Leipheimer J, Balter M, Chen A, Maguire T, Yarmush ML. Design and Evaluation of a Hand-held, Automated Venipuncture Device Employing a Force Sensing/Puncture Detection System. 2018 Biotech Symposium, Busch Campus, Rutgers, June 2018.

Leipheimer J, Balter M, Chen A, Maguire T, Yarmush ML. Design and Evaluation of a Hand-held, Automated Venipuncture Device Employing a Force Sensing/Puncture Detection System. 2018 National Biomedical Engineering Society (BMES) Conference, Atlanta, GA, October 2018.

PATENTS

Leipheimer J. Ultrasound Guided Alignment and Insertion of Percutaneous Cannulating Instrument. Provisional Application 62/743,283 filed in RU Docket no. 2018-148; LD Ref: ORC 3.8F-002

AWARDS

Rutgers 1st Place Winner – Three Minute Thesis (3MT) 2018
Rutgers 2nd Place Best Poster – 2018 Biotech Symposium
Rutgers Eugene V. Dubois Graduate Fellowship Award 2016-2017
Rutgers SUPER Grad Fellowship Award 2016-2017

JEFFERY LUO
Advisor: Ki-Bum Lee

Tissue engineering as a means of supplementing, replacing, or augmenting biological systems in patients has gathered attention as a promising avenue to treating some of the most pressing conditions in developed nations, such as cardiovascular disease and various neurodegenerative diseases. The current tissue engineering paradigm consists of deriving functional cells, addition of various cell behavior cues to elicit desired tissue activity, integration onto scaffolds (physical constructs that contribute to the function of an engineered tissue), and transplantation into a patient. Nanofibers are an attractive scaffold subtype which can be fabricated to mimic nano-scale fibers naturally found in the body while providing a high surface area to volume scaffold for cell seeding and promoting anisotropy to assist in the function of various target tissues such as bone, muscle, and nerves. Additionally, various research groups have noted that cells are capable of detecting physical nanotopography cues, which in turn affect key cellular processes such as proliferation and differentiation. While electrospinning has become one of the most common nanofiber production methods, incorporation of cell behavior cues (e.g. proteinaceous growth/differentiation factors) is limited by dehydration, shear stresses, and high electrical potential differences inherent to the electrospinning process. While the inclusion of behavioral cues into cell culture media is an acceptable means of manipulating cells *in vitro*, such an approach may not be feasible *in vivo*. Developing a benign means of including sensitive biological molecules onto nanofiber substrates would permit greater control over post-implantation cell behavior.

The goal of this research is to develop a means of adsorbing stem cell differentiation cues onto nanofiber substrates to eliminate the need to add cell behavior cues into cell culture media. Nanomaterials such as graphene are a promising avenue to accomplish this goal as they generally have high surface area to volume ratios due to their atom-thin morphology. Graphene oxide (GO) and manganese dioxide (MnO_2), two biocompatible nanomaterials capable of adsorbing proteins, are

investigated for their potential as a nanofiber surface coating to deliver stem cell differentiation factors. Gelatin, derived from irreversibly denatured collagen, is selected as the material for nanofiber electrospinning due to its biocompatibility, biodegradability, availability, and ability to adsorb aforementioned nanomaterials. Specific aims and goals of this project include: (1) synthesis of nanomaterials and aligned gelatin nanofibers for substrate assembly and characterization of protein loading capacity using various model proteins, (2) induction of stem cell alignment via nanofiber topography and differentiation via adsorbed myogenesis/osteogenesis cues, and (3) demonstrate extension of technology by using nanomaterials to localize exogenous differentiation factors to reduce off-targeting effects in an *in vivo* model.

Data gathered thus far have been oriented towards substantiating goals (1) and (2). Nanofiber-nanomaterial substrate synthesis and fabrication methods have been finalized and verified under scanning electron microscopy (SEM). High quality image collection under field emission-SEM and fiber alignment analysis via ImageJ is underway. Protein loading capacity for GO-coated and MnO_2-coated substrates have been determined for 5 different proteins, 2 of which assist in the induction of myogenesis. *In vitro* myogenesis and osteogenesis experiments have shown that adsorption of differentiation cues onto the seeding substrate is more effective than adding the same quantity into cell culture media. This is presumably due to greater delivery efficiency than allowing the differentiation factors to diffuse throughout the volume of cell culture media. Selection of GO or MnO_2 as an effective coating material was determined to be highly dependent on the properties of the differentiation cue, such as hydrophobicity. Future animal experiments would ideally focus on reducing off-targeting effects of injected cell behavior cues co-administered to assist wound healing. For example, BMP-2 is administered to induce osteogenic differentiation of mesenchymal stem cells and promote the formation of bone. An unintended consequence is ectopic bone formation near the injection site. Localization of BMP-2 onto the surface of the substrate may reduce the incidence of ectopic bone formation as it would be made more readily available to nearby implanted cells (the intended targets) at a higher local concentration.

PRESENTATIONS

Luo J, Yang L, Shah S, Lee KB. Incorporation of a Photothermal Heating Mechanism into Poly (lactic-*co*-glycolic acid) Microparticles. Northeast Bioengineering Conference, Binghamton, New York, April 7, 2016.

AWARDS

New Jersey Spinal Cord Research Fellowship (Applied) 2019
Aresty Undergraduate Research Fellowship 2015-2016
Rutgers Presidential Scholarship 2012-2016

ILEANA MARRERO
Advisor: Martin Yarmush

Osteoarthritis (OA), the principal source of physical disability and impaired quality of life in the US, is a chronic age-related disease characterized by the progressive destruction of articular cartilage, leading to

total joint deterioration. OA severely burdens the US healthcare system with overall costs of ~ $190 billion/year. Recent evidence suggests that inflammatory cytokine and chemokine release signals and cellular infiltration ultimately lead to matrix degradation and cartilage destruction. There is currently no cure for OA. Existing treatments alleviate symptoms initially; however, they are not able to alter disease progression and disease development eventually proceeds. Therefore, there is a need to develop effective therapies that could alter OA progression and promote healing in osteoarthritic joints.

One approach to alter the progression of OA has been intra-articular administration of mesenchymal stromal cells (MSC) which secrete anti-inflammatory and regenerative factors that could alter the underlying pathophysiology of OA. However, these cells are required in large numbers and are not long-lasting when freely administered. We have previously demonstrated that encapsulation of MSC lengthens their survival and promotes their secretory function, a characteristic that could serve as long term treatment for OA. On the other hand, to develop effective therapies, comprehensive *in vitro* systems that recapitulate the joint environment are needed. However, most OA-based *in vitr*o systems consist of chondrocytes, the sole cell component of cartilage, in different culture configurations while ignoring other cell components, such as synoviocytes, and the effects of cell-cell interactions. Furthermore, most in vitro systems focus on the effect of only one chondrocyte response to inflammatory stimuli, even though regenerative potential and tissue degeneration are also key components to OA progression. Therefore, an *in vitro* culture system that allows for the co-culture of multiple cell types, cell-cell interactions and cell responses, would significantly benefit the field.

In order to work towards ascertaining whether intra-articular injection of encapsulated MSC can provide sustained reduction of OA mediated joint inflammation and destruction and promote re-growth and healing, we are developing an optimized eMSC therapy that is both anti-inflammatory and chondrogeneic and testing its efficacy in an *in vitro* relevant model of OA. Our results thus far include: 1) the establishment of a chondrocyte model that can be used to evaluate inflammatory, regenerative and degradation changes in reponse to known progressive OA mediators, interleukin (IL)-1 or IL-1/TNF-α and 2) the design, fabrication, and testing a stackable culture device that can evaluate these responses in multi-culture configurations.

Using our chondrocyte model, we determined that IL-1 and IL-1/TNF stimulation upregulated the expression of pro-inflammatory cytokines associated with the pathology of OA such as IL-1β, IL-8, RANTES, MCP-1, and GM-CSF. The inflammatory stimuli also decreased chondrogenic gene expression (SOX9, COLA2, and ACAN) and upregulated ECM remodeling genes (MMP1 and MMP13). Interestingly, free MSC/eMSC treatment did not downregulate pro-inflammatory and ECM remodeling gene expression and did not significantly upregulate chondrogenic gene expression. These unexpected results led to the evaluation of prostaglandin E2 (PGE2) secretion by MSC and the assessment of PGE2 effects on chondrocytes. PGE2 is a potent immunomodulator capable of changing pro-inflammatory macrophages to anti-inflammatory macrophages and it is widely secreted by MSCs. As expected, MSC and eMSC produce high levels of PGE2; however, unlike macrophage responses, chondrocyte respond to increasing levels of PGE2 by secreting more pro-inflammatory factors.

We hypothesize that the primary role of MSC in OA may be to attenuate macrophage and synoviocyte inflammation which will secondarily mitigate the inflammatory effect on chondrocytes. This hypothesis will be investigated comprehensively in our novel multi-culture device. We have already established its efficacy as a tissue culture platform, as three relevant OA cell types chondrocytes, macrophages and MSC were shown to be viable and functional responses were relative stable in the device. Further studies will comprehensively evaluate responses to inflammatory stimuli.

PRESENTATIONS

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment. Biomedical Engineering Society Annual Meeting, October 2018.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Society for the Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS), October 2018.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment. Biotechnology Training Program Symposium, June 2018.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Society for the Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS), October, 2017.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment. Biomedical Engineering Society Annual Meeting, October 2017.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stromal Cells for Osteoarthritis Treatment. Biotechnology Training Program Symposium, June 2017.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Society for the Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS), October 2016.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Biomedical Engineering Society Annual Meeting, October 7, 2016.

Marrero I, Gray A, Maguire T, Weinberg J, Manchikalapati D, SchianodiCola J, Yarmush ML, Schloss R, Yarmush J. Effect of Local Anesthetics on Human Mesenchymal Stromal Cell Secretion and Macrophage Immunomodulation. Biotechnology Training Program Symposium, June 2016.

Marrero I, Gray A, Maguire T, Weinberg J, Manchikalapati D, SchianodiCola J, Yarmush ML, Schloss R, Yarmush J. Effect of Local Anesthetics on Human Mesenchymal Stromal Cell Secretion and Macrophage Immunomodulation. Biomedical Engineering Society Annual Meeting, October 9, 2015.

Marrero I, Schloss R, Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Biotechnology Training Program Symposium, June 24, 2015.

Marrero I, Deipolyi A, Schloss R, and Yarmush ML. Encapsulated Mesenchymal Stem Cells for Osteoarthritis Treatment. Biotechnology Training Program Symposium, June 24, 2014.

Marrero I, Maldonado S. In vitro Studies of the Tropical Seagrass Thalassia testudinum: Micropropagation and Endophytic Fungi. 31st Puerto Rico Interdisciplinary Scientific Meeting and 46th Junior Technical Meeting, Bayamón, PR, March 12, 2011.

Marrero I, Maldonado S. In vitro Studies of the Tropical Seagrass Thalassia testudinum: Micropropagation and Endophytic Fungi. XII Symposium of the Puerto Rican Mycology Society, Aguadilla, PR, May 1, 2010.

Marrero I, Maldonado S. In vitro Studies of the Tropical Seagrass Thalassia testudinum: Micropropagation and Endophytic Fungi. XV Sigma Xi Student Poster Day, Mayagüez, PR, April 8, 2010.

AWARDS

NSF Leverage Program Travel Scholarship 2018
Aaron Shatkin Scholarship 2018-Present
SACNAS Travel Scholarship 2017
GAANN Fellowship for Personalized Medicine 2017-Present
SACNAS Travel Scholarship 2016
Celgene Catherine Pegram Fellowship 2013-2014

ILIJA MELENTIJEVIC
Advisor: Monica Driscoll

Toxicity of misfolded proteins and mitochondrial dysfunction are pivotal factors that promote age-associated functional neuronal decline and neurodegenerative disease. Accordingly, neurons invest considerable cellular resources in chaperones, protein degradation, autophagy, and mitophagy to maintain proteostasis and energy/redox balance while avoiding neurotoxicity. Although these neurotoxic challenges have long been considered to be cell-intrinsic, evidence now supports that both misfolded human disease proteins and mitochondria originating in one neuron can appear in neighboring cells, a phenomenon proposed to promote pathology spread. I have been documenting a previously unknown capacity of C. elegans adult neurons to extrude large (~5µM) vesicles that include substantial amounts of cytoplasmic contents via a dynamic process requiring specific cytoskeletal proteins and motors. These exopher vesicles can include fluorescent GFP or mCherry, DiI loaded from the outside environment, aggregated human proteins such as an expanded Q128 polyglutamine protein, lysosomes, and/or mitochondria. Aggregation-prone proteins and oxidized mitochondria can appear preferentially segregated into exophers, and neurons that extrude exophers generally function better than those that do not. Inhibiting chaperone expression, autophagy or the proteasome, as well as compromising mitochondrial quality, enhances exopher prevalence, and some extruded exopher contents can be found in remote cells. Together our observations suggest exopher-genesis as a potential "garbage-removal" response to stresses in proteostasis and organelle maintenance. Our working model is that exophers are components of a conserved mechanism that constitutes a fundamental, but formerly unrecognized, branch of neuronal proteostasis and mitochondrial quality control.

As a previously undescribed phenomenon, I want to continue characterizing the exopher and investigate its function on several fronts. We were recently successful in visualizing exophers using electron microscopy. This revealed the presence of endoplasmic reticulum and lysosomes in exophers. There

appears to be two classes of extruded lysosomes; ones that appear to occupy the entire area of the exopher, and smaller lysosomes occupying a small portion of the exopher. This begs the question of how the exopher pathway interacts with the autophagy-lysosome system. It is possible that lysosomes initially make up a large portion of the exopher and shrink in size, or that these are two separate categories of lysosomes. It is possible that the extruded lysosomes are likewise dysfunctional ones being removed, or that the lysosomes being removed are functional and continue degradation in the exopher. I am utilizing time-course microscopy to study the extrusion of lysosomes in a time dependant manner to see if the extruded lysosomes shrink over time. This will also allow me to also study if exopher loaded contents are normally loaded through lysosomes. I will utilize bafilomycin A1, which should inhibit aggregate loading into lysosomes, and see if lysosomes and aggregates still localize to exophers. I will also utilize NH_4CL to disrupt lysosomal PH, to see if dysfunctioning lysosmes are preferentially loaded into exophers.

Considerable excitement in the field of neurodegenerative disease has focused on the findings that mammalian neurons can extrude conformational disease proteins as well as mitochondria. Recently I identified ced-1, ced-6, and ced-7 to act in a phosphatidylserine-independent mechanism through which exophers are engulfed by the surrounding tissue. These genes have recently been shown to be involved in the transfer of polyq protein aggregates from neurons to glia in Drosophila through an unidentified manner. Finding the protein that mediates ced-1 recognition of exophers is of great interest and may potentially have therapeutic value. Looking through known binding partners of the mammalian ced-1 ortholog CD91, I found the promising candidate GRP94/GP9630, an Hsp90 family chaperone with C. elegans ortholog enpl-1. Investigations of enpl-1 RNAi knockdown show the same phenotype of increased numbers of cells with multiple exophers as ced-1/ced-6. I have obtained a CED-1::GFP reporter and have seen that CED-1 can localize around exophers. I have also obtained a native promotor ENPL-1::GFP reporter and can find ENPL-1 localization in our neurons of interest, which has not been reported previously. I cannot observe any membrane localization of ENPL-1::GFP, but this may be due to high expression levels of the transgene. I am working on making low copy number reporters that should be closer to native protein expression levels to see more accurate localization patterns. The enpl-1 mutant proved to be developmentally lethal, so I am working on constructing a cell specific enpl-1 mutant to investigate the role of a knockout and confirm it's cell specific role. I recently analyzed a panel of tagged hypodermis (the tissue that receives the exophers) proteins involved in engulfment and found that degraded exopher fragments localize to hypodermal lysosomes. Recently we collaborated with the Albert Einstein Institute and helped them fix dozens of animals at various stages of exopher formation and degradation, which are currently being imaged with electron microscopy to visualize a detailed time course of the formation and transit of exophers.

Lastly, the mechanisms involved in regulating and executing exopher formation are of great interest to understanding this phenomenon. Through targeted RNAi screening, I discovered aip-1 to act as a genetic suppressor of exopher production, and I am now looking in more detail at its role in exopher formation. In the last year, I have been validating a methodology for performing automated rapid whole genome screens that will allow for the unbiased identification of more suppressors and enhancers, as well as for whole genome epistasis experiments. We are now able to grow a high-density RNAi feeding library in a 96 well format, replace the media so that animals can develop fertilely, and induce RNAi suppression in the animals. We have worked out an image analysis pipeline that will allow us to automatically count animals and phenotypes in each well. We have refined our machine vision process and are now able to detect general fluorescence intensity changes and detect RNAi treated and control wells, and have performed a quarter genome screen as an initial validation. We can also detect through machine vision digested exopher fragments, and can quantify what fraction of animals in a well had an exopher on

earlier days. We are now also able to detect exophers themselves by taking advantage of the cargo specificity of exophers, which gives us a direct readout of exopher occurrence and will let us screen for cargo segregation defects as well. We hope to launch a whole genome screen by the end of the year, and then subsequently perform high throughput epistasis analysis between our top hits to help us further elucidate the exopher formation and clearance mechanisms.

PRESENTATIONS

Melentijevic I. Neuronal Exophers: A Novel Mechanism for Removal of Neurotoxic Cytoplasmic Components. Plenary Talk at the C.elegans International Meeting, Los Angeles, CA, June 4, 2017.

Melentijevic I. A novel high-throughput whole-genome RNAi screening technology utilized to investigate the molecular pathways of exopher production. C.elegans International Meeting, Los Angeles, CA, June 3, 2017.

Melentijevic I. Touch neurons can toss out mitochondria. C.elegans International Meeting, Los Angeles, CA, June 3, 2017.

Melentijevic I. Structural Components and Genetic Requirements of Exophers and their Formation. 2017 C.elegans International Meeting, Los Angeles, CA, June 3, 2017.

Melentijevic I. Neuronal Exophers: A Novel Mechanism for Removal of Neurotoxic Cytoplasmic Components. Talk at the Aging, Metabolism, Pathogenesis, Stress, and Small RNAs in C. elegans Meeting, Madison, WI, July 10, 2016.

Melentijevic I. Neuronal Exophers: A Novel Mechanism for Removal of Neurotoxic Cytoplasmic Components. Plenary Talk at the 2015 C.elegans International Meeting, Los Angeles, CA, July 26, 2015.

Melentijevic I. Neuronal Exophers: A Novel Mechanism for Removal of Neurotoxic Cytoplasmic Components. Talk at the Aging, Metabolism, Pathogenesis, Stress, and Small RNAs in C. elegans Meeting, Madison, WI, July 10, 2014.

Melentijevic I. Neuronal Exophers: A Novel Mechanism for Removal of Neurotoxic Cytoplasmic Components. C.elegans International Meeting, Los Angeles, CA, July 26, 2013.

Melentijevic I. Neurite Sprouting and Synapse Deterioration in the Aging C. elegans Nervous System. Aging, Metabolism, Pathogenesis, Stress, and Small RNAs in C. elegans Meeting, Madison, WI, 2012.

PATENTS

Melentijevic I. A Novel Microscope Slide Apparatus for Accurate Temperature Control. Submitted Patent, 2012.

AWARDS

Aaron Shatkin Scholarship 2017
F31 Understanding the Exopher: A Novel Mechanism for Extrusion of Neurotoxic Contents 2017

Teaching Assistant and Graduate Assistant Professional Development Fund 2017
Understanding the Exopher: A Novel Mechanism for Extrusion of Neurotoxic Content NIH R01 Grant #8896229 2013
Rutgers Excellence Fellowship 2013-2014

YOLIEM S. MIRANDA ALARCON
Advisor: David Shreiber

A common modality in tissue engineering is to take naturally occurring molecules and proteins like collagen and supplement them with other moieties to display additional properties. With this in mind, we have been developing modified collagen materials by attaching peptides and small molecules to the lysine residues in collagen type-I. Following this rationale, I have been working with two modifications of collagen: (1) grafting methacrylic acid for properties of thermoreversibility and photoactivity; and (2) grafting beta lactam antibiotics for antibacterial protection.

In the first case, we have modified type-I collagen by adding methacrylate groups to lysine residues to develop collagen methacrylamide (CMA), which is a unique material for 3D printing scaffolds. Like collagen, CMA self-assembles into D-banded, fibrillar hydrogels, is degraded by metalloproteinases, and maintains natural bioactivity, but CMA is also thermoreversible and photoactive. CMA can reversibly form a fibrillar hydrogel at 37^{o}C and a liquid suspension at 4^{o}C. CMA can achieve a higher shear modulus, three times that of collagen type-I, after photocrosslinking. We are currently exploring the use of CMA as a bio-ink for 3D-printing of cellular scaffolds that allow for controlled differentiation of mesenchymal stem cells. To this end, we have established CMA crosslinking conditions that allow for cell encapsulation with good cell viability as well as crosslinking potential. We have identified photoinitiator concentration as critical, limiting reagent for this application.

In the second project, we have investigated covalent coupling of antibiotics from the beta lactam family, which have carboxylic acid functionalities, to the collagen backbone. Our goal is to develop a hydrogel wound dressing with enhanced anti-bacterial properties for treatment of burn wounds, chronically non-healing ulcers, and other skin disorders. A number of commercially available beta lactam antibiotics – ticarcillin, carbenicillin, ampicillin, and penicillin G – have been grafted to collagen. From this selection, carbenicillin and ticarcillin demonstrated the strongest inhibition of E. coli when covalently attached to collagen. However, there are lingering concerns that free antibiotic remains after purification steps, and that this free antibiotic is responsible for the observed inhibition. Accordingly, we are investigated different purification schemes, including size exclusion chromatography, as well as extensive rinsing of the gel to confirm that the bacterial inhibition is due to the grafted collagen.

PRESENTATIONS

Alarcón YSM, Jazwinska D, Shreiber DI. Development of Bioactive, Thermoreversible, and Photoactive Collagen Based Scaffolds for Tissue Engineering Applications. 2018 BMES Annual Meeting, Atlanta, Georgia, October 17-20, 2018.

Alarcón YSM, Jazwinska D, Shreiber DI. Development of Bioactive, Thermoreversible, and Photoactive Collagen Based Scaffolds for Tissue Engineering Applications. SACNAS- The national Diversity in STEM Conference, San Antonio, Texas, October 10-13, 2018.

Alarcón YSM, Jazwinska D, Shreiber DI. Development of Bioactive, Thermoreversible, and Photoactive Collagen Based Scaffolds for Tissue Engineering Applications. Innovations of Dermatological Sciences, Isle, NJ, October 8-9, 2018.

Alarcón YSM, Shreiber DI. Development of Bioactive, Thermoreversible, and Photoactive Collagen Based Scaffolds for Tissue Engineering Applications. SACNAS- The national Diversity in STEM Conference, Salt Lake City, Utah, October 19-21, 2017.

Alarcón YSM, Pasikanti K. GSK-687: A new tool in the RIPK1 inhibitor toolbox for chronic studies. GSK Biology Community Day, Upper Providence-Collegeville, PA, October 26, 2016.

AWARDS

PreDoctoral Leadership Development Academy-PLDA 2018-2019
National Science Foundation-Graduate Research Fellowship Program 2017-2022

JENNA NEWMAN
Advisor: Andrew Zioza

In recent years, immunotherapy has emerged as a new class of cancer treatment that has resulted in unprecedented long-term remissions in a subset of patients previously refractory to therapy. Despite clinical success, further research is needed to investigate the causes of resistance to immunotherapy, and to develop combinatorial therapies that convert progressors to responders. One strategy to augment anti-tumor immunity may be to harness bacterial and viral infection—and the resultant anti-pathogen immune response—to stimulate an anti-tumor immune response. Intratumoral injection of inactivated bacteria—called "Coley's toxin"—was first reported to initiate tumor regression in a subset of patients over a century ago. Our laboratory is revitalizing the concept of employing pathogens as an immunotherapy, investigating the mechanisms by which anti-pathogen immune responses can augment anti-tumor immunity. To this end, I am studying the effect of murine influenza (PR8/H1N1/A) infection on B16 F10 melanoma tumor growth and anti-tumor immunity in C57BL/6 mice. Mice concomitantly challenged with lung-homing B16 F10 (injected intravenously) and influenza exhibit decreased tumor burden in the lungs relative to that observed in influenza-naïve counterparts. Halted tumor growth in concomitantly challenged mice were observed to harbor a greater percentage of cytotoxic T lymphocytes in the lungs harboring specificity against tumor antigens, which possess the ability to produce tumor-killing granzymes and perforin. Given these data suggesting that influenza infection at the tumor site stymies tumor growth by promoting a potent anti-tumor T cell response, I sought to determine whether this treatment could be adapted for clinical use. The effect of influenza infection on tumor growth was subsequently tested in the form of intratumoral injection of melanoma, a more clinically feasible model of the aforementioned experiment. Contrary to the experiment conducted in the lungs, injection of live influenza into the tumor did not lead to tumor regression (possibly due to differences in virus tropism and replication in lung versus skin tissue). In an effort to release a large volume of viral antigen and initiate an anti-tumor immune response (similar to that reported with Coley's toxin), influenza virus was heat-inactivated and injected into the tumor, leading to tumor regression. Towards adapting heat-inactivated influenza to a clinical setting, I next tested the ability of FDA-approved seasonal influenza vaccines to halt tumor growth when administered intratumorally.

Influenza vaccines without an adjuvant were successful in curbing tumor growth; however, a vaccine harboring the adjuvant MF59—FLUAD—did not exhibit efficacy in halting tumor growth. The specific aims of my dissertation research are to 1) determine the mechanism by which inactivated, but not live, influenza slows tumor growth, 2) understand the immunological mechanisms driving tumor regression in mice vaccinated with Flucelvax (un-adjuvanted), and 3) investigate why FLUAD does not halt tumor growth. Significant advances have been made in addressing these aims. Experiments are currently being conducted to test the hypothesis that elevated antigen presentation may be contributing to initiation of an anti-tumor immune response in tumors injected with inactivated, but not live, virus. Analysis of Flucelvax-vaccinated tumors by flow cytometry has indicated that there is an influx of immune cells, particularly anti-tumor reactive cytotoxic T lymphocytes, into the tumor—features that are clinically correlated with responsiveness to immunotherapy. Furthermore, Flucelvax vaccination increases transcription of PD-L1, a marker of T cell exhaustion,that can render tumors susceptible to clinically available anti-PD-L1 immunotherapy. In contrast, the ability of FLUAD to induce tumor regression may be due to the influence of intratumoral B regulatory cells in the tumor; recent flow cytometry experiments have shown that FLUAD-vaccinated tumors have a significantly greater proportion of these inhibitory cells as compared to that observed in Flucelvax-vaccinated tumors. Future work will focus on further elucidation of these mechanisms, with the ultimate goal of translating this work to the clinic.

PRESENTATIONS

Newman JH, Huelsmann E, Broucek J, Kaufman H, Zloza A. Local but not distant viral infection improves cancer outcomes: implications for cancer immunotherapy. Society for the Immunotherapy of Cancer (SITC) 2016, National Harbor, MD, November 12, 2016.

Newman JH, Li S, Chesson CB, Schenkel JM, Silk A, Zloza A. Growth of Established Tumors is Reduced in Hosts Concomitantly Challenged with Non-oncogenic Acute Viral Infection. Sino-American Pharmaceutical Association (SAPA) Symposium: Revolution in Cancer Treatment-Immunotherapy and Beyond, Rutgers University, Piscataway, NJ, April 8, 2017.

Newman JH, Li S, Chesson CB, Schenkel JM, Silk A, Zloza A. Non-oncogenic Acute Viral Infection Reduces Tumor Growth in Hosts with Established Cancer. Immunology 2017, Washington, D.C. May 15, 2017.

Newman JH, Li S, Chesson CB, Schenkel JM, Silk A, Zloza A. Concomitant Non-oncogenic Viral Infection Harbors Dual Roles in Tumor Progression. The 2017 Annual Retreat on Cancer Research in New Jersey, Rutgers University, New Brunswick, NJ, May 25, 2017.

Newman JH, Chesson CB, Zloza A. Non-oncogenic Acute Viral Infection Modulates the Innate Immune Response and Reduces Tumor Growth in Hosts with Established Cancer. Society for the Immunotherapy of Cancer (SITC) 2017, National Harbor, MD, November 10, 2017.

Newman JH, Aspromonte SM, Bommareddy PK, Aboelatta M, Herzog NL, Zloza A. Influenza infection decreases tumor burden in the lungs and promotes anti-tumor immune responses. MBGSO Graduate Student Symposium, Rutgers University, Piscataway, NJ, March 23, 2018.

Newman JH, Aspromonte SM, Zloza A. Influenza infection curtails growth of melanoma in the lungs and induces anti-tumor CD8+ T cells. National Graduate Student Symposium, St. Jude Children's Research Hospital, Memphis, TN, March 27-29, 2018.

Newman JH, Chesson CB, Aspromonte SM, Bommareddy PK, Pepe R, Tarabichi S, Li S, Jhawar SR, Herzog NL, Aboelatta M, Kaul E, Estupinian R, Kane M, Silk A, Zloza A. Utilizing the 2017-2018 seasonal influenza vaccine as a treatment for cancer. Immunology 2018, American Association of Immunologists, Austin, TX, May 4-8, 2018.

Newman JH, Chesson CB, Aspromonte SM, Bommareddy PK, Pepe R, Tarabichi S, Li S, Jhawar SR, Herzog NL, Aboelatta M, Kaul E, Estupinian R, Rudra J, Kane M, Silk A, Zloza A. Intratumoral administration of the seasonal influenza vaccine halts tumor growth and reverses the immunosuppressive tumor microenvironment in mice. Regeneron Science to Medicine Forum, Tarrytown, NY, June 22, 2018.

Newman JH, Chesson CB, Aspromonte SM, Bommareddy PK, Pepe R, Tarabichi S, Li S, Jhawar SR, Herzog NL, Aboelatta M, Kaul E, Estupinian R, Rudra J, Kane M, Silk A, Zloza A. Intratumoral administration of the 2017-2018 seasonal influenza vaccine halts tumor growth and provides protection from subsequent influenza challenge. Biotechnology Training Program Annual Symposium, Piscataway, NJ, June 25, 2018.

AWARDS

Second Place in Oral Presentation Molecular Biosciences Graduate Student Symposium March 2018
First Place in Poster Presentation Biotechnology Training Program Annual Symposium June 2018

Rutgers University Graduate School Conference Travel Award for the American Association of Immunologists (AAI) Immunology 2017 Conference March 2017

Excellent Poster Award Sino-American Pharmaceutical Association (SAPA) Symposium: Revolution in Cancer Treatment-Immunotherapy and Beyond April 2017

Gallo Award for Scientific Excellence, The 2017 Annual Retreat on Cancer Research in New Jersey, Rutgers University May 2017

Princeton University International Internship Program Award Recipient,
Karolinska Institute, Department of Medical Biochemistry and Biophysics July-August 2013

BRANDON NEWTON
Advisor: Joseph Freeman

Unintentional traumatic injury remains the leading cause of traumatic muscular injury in the United States for citizens under the age of 44. The current gold standard for treating these injuries is the use of autografts, which can create pain and damage at the donor site. The use of stem cells to heal these injuries would mitigate the need for autografts saving the patient pain and potential reduced mobility due to muscle tissue extraction from healthy sites. The focus of this project is to use specially formulated electroactive hydrogels to convert mesenchymal stem cells (MSC) into myoblasts without the use of exogenous growth factors, while using both mechanical and electrical stimulation to enable directed muscle development. To date I have modified Poly(ethylene glycol) diacrylate (PEGDA) hydrogels with the positively charged molecule 2-(methacryloyloxy) ethyl-trimethylammonium chloride to form a hydrogel that is compatible with C2C12 mouse myoblasts cells and which actuates in an electric field. I am also adding decellularized muscle to the PEGDA hydrogels to promote the

differentiation of MSC into myoblasts. Preliminary tests have shown that there is a difference in the differentiation of rabbit bone marrow-derived MSC (BMSC) when exposed to factors from the muscle, the morphology resembles a myoblastic lineage. The next phase is fully characterize the differentiated cells and to treat the newly formed muscle with electrical and mechanical stimulation to form in vivo-like muscle spindles which will be studied for tensile strength. Our hypothesis based on previous results is that these materials and techniques will have a synergistic effect in promoting greater and more uniform myoblastic differentiation morphologies which may eventually be used for grafts.

AWARDS

IMSD Training Program 2017-2018

EVELYN OKEKE
Advisor: Kiran Madura

The ubiquitin proteasome pathway (UPP) is the primary mechanism for removal of cellular proteins and is conserved from yeast to humans [1]. The main focus of attention of the Madura lab is to determine how proteolytic substrates are targeted to the proteasome. In 2002, the laboratory reported evidence that there are proteins that can bind and deliver polyubiquitinylated substrates to the proteasome, and characterized these as shuttle-factors [2]. Interestingly, recent studies from our lab show that most proteasomal substrates are nuclear, whereas the proteasome is located in cytosol [3]. Consequently, Rad23 must function as a shuttle factor that transports nuclear substrates out of the nucleus to the cytosolic proteasome. Despite extensive studies by us and others, it is still unclear if shuttle factors promote this function.

The focus of my efforts is to characterize this transport mechanism. I am using unique yeast mutants with which I have been able to control Rad23 subcellular localization. Using these mutants has allowed me to test specific predictions. First, nuclear-localized Rad23 should be bound to polyubiquitinylated substrates of the proteasome, whereas cytosolic-localized Rad23 should bind the proteasome. Second, the nuclear export pathway must be a key component responsible for the translocation of substrates from the nucleus to the cytosol.

The availability of genetic mutants has facilitated studies to test these questions, and we are beginning to improve our understanding of the function of shuttle factors. One of these mutants is *sts1-2* that exhibits a proteasome localization defect at non-permissive temperature. As a result, nuclear substrates become stabilized and Rad23 is trapped in the nucleus. The other mutant is the temperature sensitive mutant called rna1-1. Rna1p is a RanGAP protein of the family of GTPases that facilitates the conversion from GTP to GDP, which is essential for the nuclear import and export cycle to occur. In rna1-1 at non-permissive temperature, nuclear substrates are also stabilized, but Rad23 is trapped in the cytosol. Subsequently, we determined that Rad23 formed a strong interaction with polyubiquitinylated substrates only when it was trapped in the nucleus. In contrast, Rad23 interaction with polyubiquitinylated proteins was strongly reduced in the cytosol.

To confirm that Rad23 binds polyubiquitinylated substrates differently based on its subcellular location, I want to examine the interaction of Rad23 with one particular nuclear substrate. It was reported that the HO protein, an endonuclease that facilitates the mating type switch in yeast and a nuclear substrate of

the proteasome, requires Ddi1 for degradation. Ddi1 is one of three known shuttle factors in yeast. The other two being Rad23 and Dsk2. To explore the idea that Rad23 and Dsk2 function have an overlapping role with Ddi1, I performed an immunoprecipitation experiment of Flag-tagged Rad23, Ddi1 or Dsk2 co-expressed with GFP-HO protein. I found that more HO was pulled down with Rad23 than with other two shuttle factors, Ddi1 or Dsk2. Using the genetic mutant sts1-2 and rna1-1, I found that HO also becomes stabilized although in different subcellular location at non-permissive temperature. In sts1-2, HO becomes stabilized inside the nucleus, while in rna1-1 HO forms aggregates in the cytosol. These finding enable me to use HO as nuclear substrate for binding studies with Rad23 in sts1-2 and rna1-1.

To support the genetic studies, I have generated recombinant proteins that can be selectively targeted for degradation using the Auxin-inducible degradation (AID) system. Specifically, AID-tagged Sts1 can be degraded in the presence of plant based protein Tir1 and the addition of auxin to the growth medium. This construct will allow me to replicate my findings in the sts1-2 mutant. A similar construct is generated for Rna1. In addition, I can reevaluate Rad23 localization and interactions with polyubiquitinylated substrates, as well as the proteasome. An extension of this study using the AID-tagged Sts1 expressed in an rna1-1 rad23Δ strain should allow me to demonstrate the nucleo-cytoplasmic trafficking of Rad23 for the first time. However, I will also investigate if the other shuttle factors, Ddi1 and Dsk2, function in a similar way.

References:

[1] Reed SH, Gillette TG. 2007. Nucleotide excision repair and the ubiquitin proteasome pathway – Do all roads lead to Rome? DNA Repair 6(2): 149-156.
[2] Chen L, Madura K. 2002. Rad23 promotes the targeting of proteolytic substrates to the proteasome. Molecular and Cellular Biology. 22(13):4902-4913.
[3] Dang FW, Chen L, Madura K. 2016. Catalytically active proteasomes function predominantly in the cytosol. J. Biol. Chem. 291(36): 18765-18777.

PRESENTATIONS

Okeke EI, Madura K. Characterization of the nucleocytoplasmic trafficking of Rad23, a shuttle factor that functions in protein degradation. The Ubiquitin Family CSHL, Cold Spring Harbor, NY, April 18-22, 2017.

Okeke EI, Madura K. Characterization of the nucleocytoplasmic trafficking of Rad23, a shuttle factor that functions in protein degradation. SACNAS, Salt lake City, UT, October 19-21, 2017.

AWARDS

NSF GRFP Fellowship 2015-2017

ANTON OMELCHENKO
Advisor: Bonnie Firestein

Traumatic brain injury (TBI) is one of the leading causes of morbidity and mortality for individuals under 45 years of age, and there is no treatment available for TBI-induced cell damage in the brain. Diffuse axonal injury (DAI), a common TBI pathology, results from severe axonal strain following primary insult. Following injury, the axonal cytoskeleton breaks down, slowing axonal recovery by interrupting axonal

protein and organelle trafficking. Mitochondrial dysfunction plays a major role in the cellular damage that occurs during subsequent secondary injury. Excessive glutamate release by neurons and glia, mechanosensitive channel activation, and the disruption of the axonal plasma membrane as a result of injury result in an increase in calcium influx into neurons. Mitochondrial buffering of the excess calcium present in the intracellular space leads to increased production of reactive oxygen species, an excess in mitochondrial fission, decreased ATP production, and the release of pro-apoptotic factors. These processes ultimately lead to cell death and contribute to the symptoms of secondary injury in TBI. As such, understanding the etiology and progression of complex neuropathologies, such as TBI, can be extremely difficult. These studies generally use expensive *in vivo* animal models, the results of which can be tedious and difficult to analyze and which require a large number of animal subjects for histopathological tissue evaluation. Other studies use *in vitro* cell culture models, which can fail to recapitulate the structure, function, architecture, or physiology of neural tissues *in vivo*. The absence of *in vitro* models of complex circuit disorders that are 1) realistic, 2) reproducible, 3) amenable to high-content data acquisition, and 4) allow for precise control of injury and cell treatments has resulted in a major bottleneck that prevents vertical advancement, largely due to the difficulty in elucidating the cellular mechanisms of CNS disorders.

The broad objectives of this project are to 1) develop a microfluidic platform to accurately model DAI *in vitro* and 2) apply developed a microfluidic platform to screen and evaluate possible therapeutics for DAI. The specific aims of this project are to 1) identify effects of stretch/strain axonal injury on mitochondrial morphology and dynamics, 2) evaluate efficacy of pharmacological agents targeting the molecular cascades of axonal stretch injury on axonal morphology, 3) evaluate efficacy of pharmacological agents targeting the molecular cascades of axonal stretch injury on mitochondrial morphology following injury, and 4) assess efficacy of screened agents on attenuation of the behavioral and neuropathological deficits associated with TBI *in vivo.*

To improve the process of DAI drug discovery, we developed a microfluidic platform to model DAI *in vitro.* Our platform uses microchannels to confine axonal tracts from the dentate gyrus synapsing on CA3 neurons, which protrude from cultured organotypic brain slices located in separated compartments. Axonal stretch/strain injury is produced via pressurization of a cavity beneath the microchannels to deflect the culture substrate and to induce stretch/strain. Our platform aims to provide a high-throughput system for screening of potential therapeutics for DAI and to allow for monitoring of axons and axonal subcellular structures before and after stress/strain injury. As mitochondrial dysfunction and calcium overload are thought to play significant roles in axonal injury and subsequent degeneration, we hypothesized that inhibition of mitochondrial fission and of the reverse-mode of the sodium-calcium exchanger (NCX) via pharmacological treatment with dynasore and SN-6, respectively, may decrease injury pathologies following stress/strain injury. To test this hypothesis, we pretreated organotypic slice cultures with 80µM dynasore, 10µM SN-6, or vehicle before application of 15% strain injury and monitored cellular response with Mitotracker Green FM and phase contrast microscopy.

Our results show that stretch/strain injury leads to a significant increase in the size and number of focal axonal swellings (FAS). Treatment with 80µM dynasore or 10µM SN-6 before injury prevents the increase in size of FAS following injury; however, treatment with 80µM dynasore, but not 10µM SN-6, attenuates the number of FAS. We next assessed the effects of DAI on mitochondrial morphology. We found that stretch/strain injury induces a significant increase in the roundness and number of mitochondria, suggesting an increase in mitochondrial fission. Our data also show that treatment with 80µM dynasore or 10µM SN-6, but not vehicle, prevents stretch-mediated reduction in mitochondrial

area and mitochondrial major axis, and increase in mitochondrial circularity. Taken together, our results suggest that mitochondrial dysfunction plays an important role in axonal pathology following stretch/strain injury. Moreover, the formation of FAS following injury may be due to increased activity of NCX and resulting elevated cytosolic calcium. Lastly, our results indicate a possible therapeutic use for dynasore and SN-6 or agents that act in the same manner. The next goals of this project are to assess the efficacy of dynasore and SN-6 on the attenuation of behavioral and neuropathological deficits associated with TBI *in vivo.*

PRESENTATIONS

Omelchenko A, Shrirao A, Hiester L, Zahn J, Schloss R, Boustany N, Yarmush ML, Firestein B. Brain-on-a-chip for Traumatic Brain Injury Drug Discovery. 2017 Biomedical Engineering Society Annual Meeting, Atlanta, Georgia, October 20, 2017.

Omelchenko A, Shrirao A, Zahn J, Schloss R, Boustany N, Yarmush ML, Firestein B. Brain-on-a-chip for Traumatic Brain Injury Drug Discovery. 2017 Biomedical Engineering Society Annual Meeting, Phoenix, Arizona, October 11-October 14, 2017.

Omelchenko A, Shrirao A, Zahn J, Schloss R, Boustany N, Yarmush ML, Firestein B. Brain-on-a-chip for Traumatic Brain Injury Drug Discovery. Third Annual BRAIN Initiative Meeting Poster Session, Bethesda, Maryland, December 12-December 14, 2016.

Omelchenko A, Sanchez S, Cabral M, Alliger A. Environmental Enrichment and its Effect on Depressive-like Behavior in Rats. 42nd Hunter College Psychology Conference Poster Session, New York, New York, March 30, 2014.

AWARDS

Rolland S. Parker Award 2014
The Livingston Welch Award 2014
Undergraduate Research Initiative Fellow 2013-2014
Jenny Hunter Scholar 2010-2014

MISAAL PATEL
Advisor: Li Cai

Spinal cord injury (SCI) results in permanent loss of neurons and glial scar formation, leading to loss of function and paralysis below the injury site. Although there are assistive devices in the market, there are no therapeutics that promote repair and regeneration after SCI. Identification of adult neural stem progenitor cells (NSPCs) and their role in injured spinal cords have provided promising opportunities for spinal cord regeneration. However, adult NSPCs mostly generate astrocytes and oligodendrocytes and the amount of neurogenesis is not sufficient to replenish all the neurons lost due to the injury. Currently, studies are targeting glial scar or residential non-neuronal cell types (e.g., astrocytes and oligodendrocytes) with transcription factor (s) (TFs) to convert them to neurons. However, functional and locomotion improvement in these studies have mostly not been reported. In addition, inhibition of scar formation is not sufficient enough to fully recover the damage after SCI. Furthermore, there is no

study known to promote neurogenesis and inhibit glial scar formation. Thus, our goal is to develop a therapeutic that would induce neurogenesis and inhibit glial scar to allow restoration of damage circuitry for overall functional and locomotion improvement.

Our preliminary studies indicate that Gsx1 and Nkx6.1 regulate Notch1, which is active in the spinal cord during development. Gsx1 is found in the dorsal region of the spinal cord and is involved in determining interneuron fate. Alternatively, Nkx6.1 is found in the ventral region of the spinal cord and is involved in determining the fate of neurons and glia.

The specific aims of this proposal are: 1) to determine the cellular role of Gsx1 on neurogenesis and specific interneuron differentiation after SCI; 2) to determine the therapeutic effect of Gsx1 astrogliosis and locomotion recovery after SCI; and 3) to elucidate the molecular mechanism of Gsx1 on NSPCs differentiation to specific types of interneurons. For aims 1 and 2, we use lentivirus-mediated gene expression system to transduce Gsx1 or Nkx6.1, with RFP reporter, into young adult mice immediately after hemisection SCI. Lentivirus carrying only RFP gene is used as a control. Injured animals are harvested at 3, 7, 14, 35, and 56 days post-injury (DPI) and immunostained with various markers to determine cellular changes around the injection site. We found that at 3 DPI Gsx1/Nkx6.1 leads to a reduction in inflammation (CD68) and cell death (Caspase3) and increase in cell proliferation (Ki67) and NSPCs activation (Nestin and Sox2). At 14 DPI, we found that Gsx1 leads to increase in neural progenitor differentiation (DCX) and decrease differentiation in glial (e.g., astrocytes, GFAP, and oligodendrocyte progenitor, PDGFRα) lineage. In comparison, Nkx6.1 leads to an increase in the differentiation of both neural progenitor and astrocyte, but a decrease in oligodendrocyte progenitor. Furthermore, our results show that both Gsx1 and Nkx6.1 increase the number of mature neurons (NeuN+) around the injury site compared to control at 56 DPI. Detailed analysis identified that Gsx1 induces a higher number of glutamatergic neurons (vGlut2) and cholinergic neurons (ChAT) and reduces the number of GABAergic neurons (GABA) compared to control at 56 DPI; while Nkx6.1 expression increases the number of GABAergic neurons and cholinergic neurons, but not glutamatergic neurons. Furthermore, Gsx1 leads to significant reduction in astrogliosis (GFAP) and glial scar formation (CS56) around the injury site compared to Nkx6.1 and control at 56 DPI. To evaluate the functional and locomotion recovery after SCI, we assessed the locomotor function using the Basso Mouse Scale (BMS) score in open field test. The expression of Gsx1, but not Nkx6.1, promotes recovery of locomotor function after SCI.

Next, in order to elucidate molecular mechanism of Gsx1, we will perform single-cell RNA-sequencing (scRNA-seq) analysis on neural differentiation *in vitro* using a neural stem cell line (e.g., CRL-2925), followed by downstream clustering and pathway analysis. Currently, we are performing *in vitro* differentiation assay and preparing samples for scRNA-seq. Successful completion of the proposed study will provide new insights for the cellular and molecular mechanisms underlying functional recovery after SCI, which will accelerate the development of regenerative medicine for SCI.

PRESENTATIONS

Patel M, Anderson J, Lei S, Risman R, Cai L. Nkx6.1 induces neurogenesis after Spinal Cord Injury. Biomedical Engineering Society (BMES) Conference, Atlanta, GA, October 18, 2018.

Patel M, Anderson J, Lei S, Risman R, Cai L. Nkx6.1 Induces Neurogenesis After Spinal Cord Injury. Biotechnology Training Program Symposium, Piscataway, NJ, June 25, 2018.

Patel M, Anderson J, Lei S, Risman R, Cai L. Forced Expression of Nkx6.1 Induces Neurogenesis After Spinal Cord Injury. Northeast Bioengineering Conference (NEBEC), Philadelphia, PA, March 29, 2018.

Patel M, Anderson J, Lyu YL, Cai L. Epigenomic Approaches to Analyze Role of Topoisomerase II-beta on Neural Development. Biomedical Engineering Society (BMES) Conference, Phoenix, AZ, October 12, 2017.

Patel M, Cai L. Genomic Approaches to Analyze Role of Topoisomerase II beta in Neural Development. Biotechnology Training Program Symposium, Piscataway, NJ, June 8, 2017.

Patel M, Cai L. Genomic Approaches to Analyze the Role of Topoisomerase IIb in Neural Development, 43th Annual Northeast Bioengineering Conference (NEBEC), Newark, NJ, April 1, 2017.

Patel M, Kwan KY, Cai L. Genomic Approaches to Analyze the Role of Topoisomerase IIb in Neural Development. Rutgers Joint Molecular Biosciences Graduate Student Association (JMBGSA) Annual Symposium, Piscataway, NJ, April 1, 2016.

PATENTS

Cai L, **Patel M.** TREATING SPINAL CORD INJURY (SCI) AND BRAIN INJURY USING GSX1. U.S. Patent Provisional Application No. 62/721,679.

AWARDS

TA/GA Professional Development Award 2018
Associate Alumnae of Douglass College Fellowship 2018
Ruth Adams Fellowship 2018
Margaret Denton Wagner Fellowship 2018
U.S. Department of Education GAANN Fellowship 2017 – Present

XIOMARA PEREZ
Advisor: Martin Yarmush

Traumatic Brain Injury (TBI) is a leading cause of morbidity and mortality worldwide, with approximately 1.7 million new cases every year. It is characterized by an initial mechanical injury, leading to secondary physiological responses which result in tissue and cellular degeneration. Current treatments aim to stop or slow the progression of secondary injury in order to promote functional recovery. To date, there are no Food and Drug Administration (FDA) approved pharmacological therapies which adequately promote neuroprotection and/or neuroregeneration and, therefore, functional improvement is minimal, especially in severely injured patients. Given the complexity of the TBI cascade, multi-modal therapies, which combine therapeutic and regenerative effects, may be more effective in promoting functional recovery.

The objective of this project is to develop a multi-modal therapy which can attenuate neuroinflammation and promote neuroprotection and neuroregeneration, and thereby leading to enhanced functional recovery. The proposed therapy will entail alginate co-encapsulation of Mesenchymal Stromal Cells (MSC) with nanoparticles (NPs) containing Brain-Derived Neurotrophic

Factor (BDNF). MSC are a promising therapy for TBI due to their innate ability to secrete anti-inflammatory and regenerative cytokines and growth factors. Previous work in our lab demonstrated superior immunomodulatory function of encapsulated MSC (eMSC) compared to free MSC, both in vitro and in an *in vivo* Spinal Cord Injury (SCI) model, where minimal functional recovery was recorded. Thus, we are proposing to improve the therapeutic outcome of eMSC treatment via BDNF supplementation, which has been shown to be important in promoting axonal elongation and neuronal survival, and which is minimally secreted by MSC and not at all by eMSC. The project will be addressed by 1) optimizing and characterizing alginate encapsulated NPs for long-lasting, controlled delivery of BDNF, 2) co-encapsulating MSC with BDNF loaded NPs and evaluating therapeutic efficacy *in vitro*, and 3) therapeutic evaluation of co-therapy *in vivo*.

Thus far my research has demonstrated that, 1) while free MSC secrete higher levels of BDNF and GDNF compared to free eMSC, neither secrete levels of BDNF required to be therapeutic, 2) BDNF can protect neurons from H_2O_2 induced cell death, but treatment with MSC alone does not induce neuroprotection, 3) we can encapsulate albumin both in liposomes and PLGA NPs and 4)MSC can be successfully co-encapsulated with PLGA NPs and remain viable for at least 48hrs.

Thus, this year's work will focus on further characterization of MSC and BDNF co-treatment via morphology and viability changes of neurons. In addition we will optimize BDNF-NPs which will be encapsulated in alginate-microspheres and evaluated in the neuronal injury we have established. We will also focus on evaluating the neuroprotective and anti-inflammatory properties of the combinational therapy. With the acquired preliminary data, I intend to defend my thesis proposal this upcoming spring.

PRESENTATIONS

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Provides Prolonged Local Analgesic Effects In Vivo. Biomedical Engineering Society (BMES), Atlanta, Georgia, October 2018.

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Provides Prolonged Local Analgesic Effects In Vivo. Society for the Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS), San Antonio, Texas, October 2018.

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Preserves Mesenchymal Stromal Cells Anti-Inflammatory Function. Biotechnology Training Program Annual Symposium, Piscataway, New Jersey, June 2018.

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Preserves Mesenchymal Stromal Cells Anti-Inflammatory Function. Biomedical Engineering Society (BMES), Phoenix, Arizona, October 2017.

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Preserves Mesenchymal Stromal Cells Anti-Inflammatory Function. Society for the Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS), Salt Lake City, Utah, October 2017.

Perez XI, Davis M, Marrero-Berrios I, Maguire T, Schloss RS, Yarmush J, Yarmush ML. Alginate-Liposomal Bupivacaine Formulation Provides Prolonged Local Analgesic Effects. Biotechnology Training Program Annual Symposium, Piscataway, New Jersey, June 2017

Perez XI, Chieu L, Leung J, London J, Taylor BL, Freeman JW. Mechanical Reinforcement of a Tissue Engineered Bone Scaffold with the Incorporation of Hydroxyapatite (HAP) Columns and Electrodeposition Mineralization. Society for the Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS), Long Beach, California, October 2016**.**

AWARDS

SACNAS ASSIST Travel Grant 2017
School of Engineering Graduate Fellowship 2016-2017
MS-to-PhD Bridge Program Fellowship 2016 -2017
SACNAS Travel Scholarship 2016

WILLIAM PFAFF
Advisor: Michael Dunn

Osteoarthritis is one of the major causes of joint pain and disability in middle-aged and older adults. Wear and sports-related injuries cause the degeneration of articular cartilage, and may necessitate surgical intervention due to the tissue's natural inability for self-repair. Current surgical interventions include the practice of microfracture and autologous chrondrocyte transplantation. The practice of microfracture involves the debridement of damaged cartilage and piercing the bone surface to release bone marrow stem cells and blood that initiates wound healing. While the resulting scar tissue can alleviate joint pain, it is mechanically inferior to native cartilage tissue and merely delays the progression of osteoarthritis. Autologous chondrocyte transplantation is an experimental technique where chondrocytes are harvested from non-load-bearing cartilage and implanted into the defective site. While these chondrocytes are capable of proliferating and producing collagen type II, there is no organization of the fibrous extracellular matrix (ECM) and the resulting scar tissue is mechanically inferior to native cartilage. The field of articular cartilage tissue regeneration is currently examining the development of implantable scaffolds seeded with autologous chondrocytes that can develop a strong and durable ECM that is identical to native cartilage. Current models have attempted mechanically preconditioning a scaffold seeded with autologous chondrocytes in vitro prior to implantation, while other studies have tested how scaffolds with a gradient of porosity/growth factors/proteoglycan distribution can direct ECM development of chondrocytes. Our lab's focus is to determine how a scaffold's gradient mechanical properties that can condition chondrocytes to produce the desired ECM while in vivo, obviating the time and expense of preconditioning in vitro as well as the risks of using growth factors. The objective of this study is to develop a polymer fiber-reinforced composite scaffold that can be seeded with autologous chondrocytes and immediately implanted into the defect site to assist in cartilage regeneration. The central hypothesis is that since chondrocytes produce collagen type II orthogonal to the direction of compressive stress, a scaffold that undergoes increasing lateral compressive stress with depth will cause chondrocytes to produce fibers in an orientation similar to native cartilage tissue. Our rationale is that if we can prove that autologous chondrocytes can be mechanically conditioned to produce the

desired ECM in vivo, then we can create a clinically viable scaffold that is superior to current surgical interventions while avoiding the risk and cost of experimental scaffolds that require growth factors or preconditioning in vitro. The specific aims are to (1) develop a composite scaffold with the same day-zero biomechanical properties as native cartilage and is capable of internal lateral expansion when undergoing compression and shear distortion, (2) determine how this scaffold supports chondrocyte proliferation and collagen type II production and orientation in vitro, and (3) test how the scaffolds can integrate into the surrounding native cartilage tissue when laterally compressed prior to insertion in vivo. Our lab has developed a prototype scaffold consisting of woven and sintered polycaprolactone fibers with an hyaluronate-collagen substrate. We have optimized the biomechanical properties of the scaffold by testing prototypes with fibers of varying sizes and weaving patterns, as well as substrates with varying compositions of collagen and proteoglycans. We are currently isolating chondrocytes from ovine cartilage tissue for use in in vitro biocompatibility studies. Once the scaffold has the requisite biocompatibility for hosting chondrocytes, we are going to test its ability to support an autologous chondrocyte population in vivo in a rat model, and test its functional capabilities in a larger animal model. By examining the response of the chondrocyte population to the mechanical stimulus in vivo, we can provide insight on how collagen type II fibers can be produced and aligned to recreate the ECM found in native cartilage.

PRESENTATIONS

Pfaff WH, Dunn MG, Gatt C. Development of an Anisotropic Silk Scaffold for Meniscus Replacement. Biotechnology Training Program Annual Symposium, South Plainfield, NJ, June 24, 2014.

Pfaff WH, Dunn MG, Gatt C. Developing Aligned Silk Fiber Scaffolds for Orthopaedic Applications. Biotechnology Training Program Annual Symposium, South Plainfield, NJ, June 24, 2015.

Pfaff WH, Dunn MG, Gatt C. Mechanical Characterization of Anisotropic Composite Scaffold for Cartilage Defects. Biotechnology Training Program Annual Symposium, South Plainfield, NJ, June 9, 2016.

Pfaff WH, Dunn MG, Gatt C. Composite Collagen-Alginate Substrates in Biomimetic Articular Cartilage Scaffolds. New Jersey Center for Biomaterials Symposium, New Brunswick, NJ, October 24, 2016.

AWARDS

Graduated from Brown University with Honors 2010

CHRIS RATHNAM
Ki-Bum Lee

Regenerative medicine is a continually growing field that has attempted to revolutionize the healthcare industry. My research focuses on two main areas for advancing the field of regenerative medicine. I hope to 1) develop technologies to control gene activation and repression to control stem cell behavior and 2) develop inorganic nanoscaffolds to deliver stem cells to injury sites to treat CNS injuries.

Ever since Yamanaka and colleagues' pioneering work on cellular reprogramming, the use of transcription factors to modulate cell fate and behavior has exploded. However due to the safety concerns with the use of viral vectors and integrating plasmids, the potential for translation of many of these studies has been severely limited. To this end our lab has developed a novel nanoparticle based platform, termed NanoScript, that has been designed to mimic the structures and functions of natural transcription factors. Using NanoScript we have demonstrated that adipose derived mesenchymal stem cells can be induced towards different cell lineages including myocytes and chondrocytes. In addition, NanoScript can be easily designed to target various genes and either upregulate or repress them. I have recently been working on methods to 1) utilize NanoScript to regenerate otic neurons to treat hearing loss and 2) increase specificity of NanoScript by utilizing triplex forming oligonucleotides (TFO) as a DNA-binding domain.

To regenerate otic neurons I have designed and constructed a NanoScript platform that mimics the Ascl1 transcription factor. This transcription factor has been shown in literature to reprogram spiral ganglion non-neuronal cells into otic neurons. Utilizing NanoScript I have tested the differentiation of IMOP cells into otic neurons. I have optimized delivery efficiency into the cells and gene expression. I have shown that NanoScript treated cells have a higher differentiation efficiency and show more mature neuronal markers such as PSD95. I am currently testing my platform in cochlear explant cultures to see the differentiation of SGNNC's into otic neurons.

To achieve the increased specificity of NanoScript I am testing the use of TFOs as the DNA binding domain. TFOs are oligonucleotides that bind to the major groove of DNA. Compared to the previous DNA-binding domain that can only recognize 6-8 base pairs, TFOs can recognize 20-22 bp making them much more specific. I have designed TFOs to target the promoter of the genes MyoD1 and ASCL1 and have tested the gene activation using NanoScript and have showed a similar or increased activation for the two genes using the TFO. In addition, I am testing the binding affinity of the TFO with various numbers of base pair mismatches using SPR to elucidate how base pair mismatches will affect the binding of the TFO. I am also looking to test the binding of the TFO's in the entire genome to find how many sites these TFOs bind to compared to hairpin polyamides. I have showed that TFO's can effectively upregulate their target genes when combined with the NanoScript platform and that adipose derived mesenchymal stem cells treated with MyoD TFO NanoScript can differentiate into muscle cells.

At the culmination of my research I hope to improve both the specificity as well as the level of activation of NanoScript to raise it to the next level where it can be used for stem cell differentiation and transplantation for the treatment of various disorders.

AWARDS

NJCSCR Fellowship 2018
Rutgers CCB Excellence Fellowship 2015
Rutgers J. Livingston Morgan Award 2015

EVE REILLY
Advisor: Mikel Zaratiegui

Epigenetic modifications can transform chromatin into two distinct states: an 'open' form known as euchromatin, or 'closed' and highly compact heterochromatin. Maintaining or switching between these chromatin states in a temporally and spatially defined manner is essential for coordinating gene regulation and ensuring genome organization and stability. Position effect variegation (PEV), first observed in Drosophila melanogaster, is an epigenetic phenomenon characterized by variable expression of a reporter gene due to stochastic heterochromatic spreading with stable inheritance of these expression patterns once they are established. We performed a genetic screen for PEV mutants in Schizosaccharomyces pombe by transposable element mediated mutagenesis. Surprisingly, integration density profiling of the mutagenized genes enriched in the screen after several rounds of selection for loss of pericentromeric heterochromatin silencing revealed a number of essential genes involved in DNA replication, recombination, and repair.

One of the biggest open questions in epigenetics is how heterochromatic marks are faithfully inherited by subsequent generations after disruption by the replication fork in the process of DNA replication and cell division. To determine whether we could generate novel alleles in essential genes involved in the DNA processes described above, we performed a secondary screen using CRISPR-Cas9 mediated mutagenesis guided by the results of the PEV screen to isolate alleles in target genes which are associated with heterochromatic silencing defects. This work is ongoing and we have identified several candidate mutant alleles to date which exhibit a variegation phenotype combined with de-repression of pericentromeric transcripts. We are currently in the process of validating that the silencing defect associated with these alleles is a direct result of the mutation and not an off-target or background effect of CRISPR-Cas9 mediated mutagenesis. After validation of these alleles, we intend to perform a more comprehensive analysis of the associated silencing defects.

Investigating the effect of sap1-c genome-wide.

Sap1 is a DNA binding protein which binds throughout the Schizosaccharomyces pombe genome, generating nucleosome free regions and influencing overall nuclear architecture (Mizuguchi et. al., 2017). Notably, Sap1 acts as a replication fork barrier within ribosomal DNA repeats, orienting the direction of replication so that it is co-directional with respect to transcription of rDNA genes (Krings et. al., 2005). An allele of sap1, sap1-c, has been described as retaining the ability to bind DNA yet losing replication fork barrier activity, leading to ectopic recombination at Tf2 long terminal repeats (LTRs) genome-wide (Zaratiegui et. al., 2011).
We are in the process of characterizing other phenotypes associated with the sap1-c allele. Previously, it has been observed that sap1-c cultures growing in rich media exhibit dramatically reduced viability upon reaching saturation, while the observed lethality upon reaching saturation is reduced in minimal media. This collection of phenotypes is characteristic of chronological life span mutants which are often studied as a model for aging in yeasts. As a preliminary experiment, we performed RNA-seq analysis on wild-type and sap1-c cultures in exponential growth (~OD .5) and upon reaching saturation (~OD 3.0) to analyze differentially expressed genes. We found that ncRNAs and rDNAs in particular increased significantly; however, there were no other obvious patterns in differentially expressed genes between each condition. Plotting transcription changes in each strain in a genome browser, however, revealed a striking pattern of downregulated genes proximal to and within the subtelomeric regions of chromosomes I and II. To test whether this pattern was a result of direct regulation of those genes by Sap1 or the product of general transcriptional changes in the subtelomeric regions, we quantified the

effect on transcription of several reporters located in the subtelomeric region of chromosome II. All three reporter strains showed decreased expression. Subtelomeric regions are typically repressed as a result of heterochromatic silencing; additionally, it has previously been suggested that Sap1-bound LTRs in the subtelomeric region of chromosome II may exhibit boundary element activity. We are currently in the process of testing the hypothesis that Sap1's replication fork barrier activity may prevent encroachment of subtelomeric heterochromatin on nearby genes by establishing and/or reinforcing chromatin boundaries. We are also interested in assessing whether altered chromatin domains as a result of loss of Sap1's replication fork barrier activity contribute to the reduced chronological life span phenotype associated with the sap1-c mutant.

Identification of variables affecting Tf2 transposable element mobilization in S. pombe.

The S. pombe genome is colonized by the Tf family of transposable elements, Tf1 and Tf2. Tf1's mobilization preferences have previously been described extensively; however, Tf1 is only active in wild isolates of S. pombe and is virtually extinct in laboratory strains, while Tf2 remains active within laboratory strains of S. pombe (Esnault et. al., 2015). A notable characteristic of Tf2 transposons is the propensity to mobilize via homologous recombination between cDNA and endogenous Tf2 elements (Hoff et. al., 1998). This dependence on HR is in stark contrast to Tf1, which primarily mobilizes via an integrase-dependent mechanism. We set out to assess the ability of ectopically expressed Tf2 to mobilize in a strain in which all 13 full length Tf2 transposable elements have been removed by CRISPR-Cas9 (referred to as "Tf-0"). We found that Tf2's mobilization efficiency is dramatically reduced in Tf-0, consistent with the loss of HR target sites due to the absence of full-length Tf2s.
Previously, our lab has demonstrated that Sap1's replication fork barrier activity is required for recruitment of Tf1 integrase and subsequent transposition (Jacobs et. al., 2015). Although Tf2 is less dependent on integrase-mediated mobilization, Sap1's replication fork barrier activity also has the potential to influence HR-mediated mobilization. We found that Tf2 exhibited significantly decreased mobilization efficiency in a sap1-c mutant background; further, in a sap1-c Tf-0 mutant background Tf2 was rendered completely unable to mobilize. We are in the process of generating a library of integrase-mediated Tf2 insertions in the Tf-0 strain and sap1-c to assess the integration preferences of Tf2 integrase and determine whether loss of Sap1's replication fork barrier activity affects Tf2 integrase targeting preferences.
Esnault C, Levin HL (2015). The Long Terminal Repeat Retrotransposons Tf1 and Tf2 of Schizosaccharomyces pombe. Microbiol Spectr 3(4).

Hoff EF, et al. (1998). Schizosaccharomyces pombe retrotransposon Tf2 mobilizes primarily through homologous cDNA recombination. Mol Cell Biol 18(11): 6839-6852.

Jacobs JZ, et al. (2015). Arrested replication forks guide retrotransposon integration. Science 349(6255): 1549-1553.

Krings G, Bastia D. (2005). Sap1p binds to Ter1 at the ribosomal DNA of Schizosaccharomyces pombe and causes polar replication fork arrest." J Biol Chem 280(47): 39135-39142.

Mizuguchi T, et al. (2017). Shelterin components mediate genome reorganization in response to replication stress. Proc Natl Acad Sci U S A 114(21): 5479-5484.

Zaratiegui M, et al. (2011). CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR. Nature 469(7328): 112-115.

PRESENTATIONS

Hardy E, Steward R, **Reilly E.** Dlg5, a MAGUK family protein, functions in Drosophila oogenesis. 53rd Annual Drosophila Research Conference, 2012.

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Hardy E, Changela N, Tan W, Steward R, **Reilly E.** Zfrp8, a conserved stem cell factor, interacts with the MAGUK family protein Dlg5. Annual Retreat on Cancer Research, NJ, 2011.

AWARDS

Anne B. and James B. Leathem Fellow 2018
Honorable Mention, NSF GRFP
Henry Rutgers Scholar Award for outstanding thesis May 2012
Paul Robeson Scholar, for completion of senior research thesis May 2012
Rutgers School of Arts and Sciences Honors Program Scholar May 2012
Highest Honors, Rutgers MBB Departmental Honors Thesis April 2012
Poster Prize (1st place), Rutgers MBB Senior Research Poster Session April 2012
Jerome and Lorraine Aresty Research Scholarship, small grant for research purposes January 2012
Rutgers Division of Life Sciences Summer Undergraduate Research Fellowship Summer 2011
Dean's list, 6 out of 8 semesters
James Dickson Carr Four Year Scholarship 2008-2012

NISHA SINGH
Advisor: Bonnie Firestein

Traumatic brain injury (TBI) is a leading cause of morbidity and death in the United States. Despite its severity and prevalence, however, there are currently very limited treatment options for this condition. Physical damage to the brain is followed by a secondary phase of injury in which cellular signaling becomes perturbed, often resulting in harmful effects. These include cellular energy failure, excitotoxicity, axonal damage, and neuronal death. The goal of my work is to understand the molecular mechanisms underlying this secondary phase of TBI. In particular, my research focuses on regulation of the Janus kinase and signal transducer activator of transcription (JAK-STAT) pathway after TBI. The JAK-STAT pathway is a crucial regulator of many cellular processes including growth, proliferation, migration, differentiation, and survival. The pathway is also involved in cytokine signaling and inflammatory response. It mediates these processes by transducing signals received at the cell surface to the nucleus, subsequently stimulating transcriptional activation of target genes. Previous studies have shown that the JAK-STAT pathway is induced in the brain after multiple types of cerebral insult, including TBI, stroke, and status epilepticus (SE). Hence, a detailed characterization of the JAK-STAT pathway after TBI will further our understanding of the molecular pathologies associated with injury. Such knowledge should facilitate the discovery of potential molecular targets for therapeutics.

Current experiments examine the induction of the JAK-STAT pathway after different severities of TBI, after repetitive injury, as well as at various time points after injury. I am analyzing induction of various JAK-STAT-associated proteins in brain tissue from mice subjected to controlled cortical impact (CCI), a robust in vivo model for TBI. One such protein is phosphorylated Stat3 (pStat3). Preliminary results demonstrate that pStat3 levels are increased in cortical tissue after repetitive injury as early as 1 hour

post-CCI. Furthermore, differences in pStat3 expression after various types of repetitive injury are also apparent. Ongoing studies examine expression levels of additional JAK-STAT-associated proteins at various times post-CCI and in other brain regions. The primary long-term goal of this project is to identify particular components of the JAK-STAT pathway that can be targeted to improve recovery after TBI.

VICTOR TAN
Advisor: Shengkan Jin

I had previously worked on a phosphoproteomics project. The project has been completed on my end and results are reported in pending publications. I will be reporting on the new project involving genetic engineering:

Traditional genome editing technologies have relied on introducing a double-stranded break (DSB) in the DNA. Subsequently, the cell repairs these breaks via a variety of repair mechanisms: nonhomologous end joining (NHEJ) and homology directed repair (HDR). Unfortunately, DSBs are difficult to control, often resulting in off-target effects. These unintended breaks are then repaired but miscues in the process result in accumulation of insertions and deletions (indels) at various locations in the genome. From a therapeutic point of view, unintended indels at loci can result in disaster if key genes were affected. In addition, HDR-dependent genome editing is dependent on the mitosis of the cell. This in turn limits the efficiency of HDR, which is further compounded by competition from NHEJ processes. As a result, it is necessary to improve on genome editing techniques in order to increase efficacy and minimize off-target effects.

Our lab utilizes a novel base editing technique that takes advantage of the advent of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). This in turn requires neither DSBs nor reliance on HDR mechanisms. Instead of introducing foreign DNA to be incorporated into the genome, we instead modify the bases directly via conversion of a C:G base pair into a T:A base pair. The technology comprises of the following modules:

1. A cytidine deaminase enzyme
2. Catalytically impaired Cas9 protein
3. Guide RNA for targeting

The aims of the project are as follows:

1. Establish and improve genome editing platform.
   Our current model employs a modular system. Initially, the guide RNA guides the Cas9 protein to the site of interest. Using RNA-based aptamers, we then recruit the various effector proteins to target sites. The current effector protein is a base editor that deaminates cytidines, creating uracil. This conversion is then fixed by the cell's repair mechanisms into a thymidine, effectively converting a C:G base pair into a T:A base pair. Because of the many modules in the platform, there are various areas we can work on in order to improve target recognition, effector function, and reduce off-target effects. We are currently looking into 1) increasing recruitment sites to enhance effector localization, 2) modifications to effector proteins to retain nuclear localization, and 3) codon-optimization of constructs to ensure proper protein expression.

2. Efficacy on disease model: DMD.
   Duchenne Muscular Dystrophy (DMD) is a disease characterized by muscle degeneration, which results in muscle weakness, motor dysfunction, and even learning disabilities. The root cause of the disease is a failure in production of a key protein dystrophin due to nonsense mutations in the gene. By using our base editing technology, we plan to modify the exon-intron junction at exon 51. This in turn leads to exon-skipping to bypass the halt of protein production and instead forcefully produce dystrophin. We plan to use disease cell models and evaluate re-expression of functional proteins through biochemical and functional assays.

3. Stop codon introduction for immunotherapy:
   The introduction of Chimeric Antigen Receptor (CAR) T-cells has been a boon for the field of immunotherapy. The construction of these CAR-Ts require genetic modification of the patient-specific T-cells. We plan to use our CRISPR platform in order to better modify the cells. Specifically, we edit key nucleotides in the host's pre-existing T-cell receptor locus to alter the corresponding codons into stop codons. By knocking out the host's pre-existing T-cell receptor repertoire, we can minimize graft vs host diseases and balance on-target efficacy with off-target toxicity. The specific modifications are to coding strand of genes containing CGA (Arg), CAG (Gln), and CAA (Gln) to create TGA, TAG, or TAA stop codons, respectively. Alternatively, we plan to target TGG (Trp) in the coding strand which in turn creates TGA, TAG, and TAA stop codons by changing any G to A (or C to T in noncoding strand).

PRESENTATIONS

Tan VM, Drake JM. Synergistic Combination of Kinase Inhibitors with Enzalutamide against Advanced Prostate Cancer. 2018 Annual Retreat on Cancer Research, New Jersey, May 25, 2018.

Tan VM, Drake JM. Enhancing Standard of Care Anti-androgen Therapies with Synergistic Addition of Tyrosine Kinase Inhibitors in Advanced Prostate Cancer. 2018 Biotechnology Training Program Annual Symposium, New Jersey, June 8, 2018.

Tan VM, Drake JM. Synergistic Combination of Kinase Inhibitors with Enzalutamide against Advanced Prostate Cancer. 2017 Annual Retreat on Cancer Research, New Jersey, May 25, 2017.

Tan VM, Drake JM. Enhancing Standard of Care Anti-androgen Therapies with Synergistic Addition of Tyrosine Kinase Inhibitors in Advanced Prostate Cancer. 2017 Biotechnology Training Program Annual Symposium, New Jersey, June 8, 2017.

AWARDS

Gallo Award for Scientific Excellence: Podium Presentation 2018
Gallo Award for Scientific Excellence: Podium Presentation 2017
Martin L. Yarmush Award: 1st place Outstanding Poster Presentation 2017
Rutgers Institute of Quantitative Biomedicine, Joint Student 2016

LIAM TURK
Advisor: Davide Comoletti

I am providing updates on the progress made in two distinct research projects that I have been involved with in the Comoletti lab. One project was underway when I joined the lab, and I had the opportunity to meaningfully contribute to the research and help finish the overall project. The second is my thesis research project that I recently proposed in my oral qualifying exam.

1) Specification and maturation of neurons rely heavily on the protein-protein interactions (PPIs) that occur within the context of the synapse. While the identities of the proteins are known, the interactome or overall network of protein connections that orchestrate aspects of brain development and function is largely untapped. Knowledge of extracellular PPIs and subsequent biochemical and structural data that highlight unique characteristics of each interaction are of high importance; they provide possible new functions and explorable pathways for proteins known to be implicated in disease and information that can be used when considering extracellular proteins as therapeutic targets within the field of rational drug design.

Using an extracellular, proteomic screen of neuronal cell surface molecules, we identified a number of new protein-protein interactions that potentially occur within the synapse. Among these, we described the interaction pattern that takes place within the IgLON protein family. The IgLON protein family consists of five members (IgLON1 – IgLON5) and they form homo and heterodimers that could span the synaptic cleft. This protein family and genetic abnormalities affecting its members have been implicated in a number of neurological diseases from Alzheimer's disease to depression. We structurally and biophysically analyzed the homo and heterodimeric interactions occurring between IgLON protein family members and also showed that these interactions occur in trans, forming an interaction between cells.

My specific contributions to this project revolved around the crystallographic analysis of the protein-protein interactions. After data collection at CHESS, I solved three distinct crystal structures ranging from 3.3Å to 4.0Å resolution that demonstrate an overall similar interaction scheme between the IgLONs. The specific structures I solved include 1) the IgLON2-IgLON2 homodimer, 2) the IgLON5-IgLON5 homodimer, and 3) the IgLON2-IgLON4 heterodimer. All three structures have been deposited to the PDB under PDB IDs 6DLE, 6DLD, and 6DLF, and they will be released upon publication.

2) Reelin is a secreted protein critical for development. Primarily expressed by Cajal-Retzius cells during pre- and early postnatal development but also by GABAergic interneurons in the adult brain, reelin is critical for proper neuronal migration, dendrite development, and synapse formation. These effects are the products of reelin's interactions with its known receptors, the very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2, also known as LRP8), but studies have demonstrated that it is likely that there are unidentified receptors that interact with reelin and produce signaling events. Abnormalities in reelin mRNA and protein levels are associated with neuropsychiatric diseases like autism spectrum disorders, schizophrenia, and depression.

I recently proposed my thesis project entitled, "Solving the structural activation of the reelin signaling pathway and expanding reelin's interaction profile." The aims of the project are as follows: 1) to characterize the full-length structure of reelin, 2) to identify previously unknown reelin binding partners, and 3) to solve the structure of a signaling competent reelin fragment in complex with its known receptors. Accomplishing these aims will provide valuable signaling, functional and regulatory data in regard to the reelin pathway and its interactions with both known and as of yet unknown receptors. It

will also be of value to the health community, as studies have considered the possibility of reelin and its receptors as therapeutic targets.

Thus far, I have been able to purify various constructs of the reelin protein in sufficient amounts for structural and biophysical experiments; preliminary cryo-EM and SAXS data demonstrate that the samples I have purified are of high quality. I have also had success in purifying the receptors, VLDLR and ApoER2 to begin to study the interaction between ligand and receptor. Receptor interaction requires the presence of the central fragment of reelin's eight repeats (RR3-6), however signal activation is dependent on the oligomerization state of reelin. It is my goal to copurify in complex, the central fragment of reelin along with ApoER2 and VLDLR and then perform cryo-EM as well as screening crystallogenic conditions. Solving the complex structure and then analyzing it relative to the structure of the apoprotein can provide information on whether or not a conformational shift is occurring; it may also provide evidence of dimeric reelin binding two receptors simultaneously resulting in receptor clustering that has indirectly been shown as a possible mechanism of activation in previous studies.

PRESENTATIONS

Turk LS, Kuang X, Patel K, Dai W, Comoletti D. Structural Characterization of Reelin Using Cryo-Electron Tomography. Rutgers Brain Health Institute Symposium, Raritan Valley Community College, December 1, 2017.

Turk LS, Kuang X, Patel K, Dai W, Comoletti D. Structural Characterization of Reelin Using Cryo-Electron Tomography. Annual Graduate Student Research Symposium, Rutgers University, New Brunswick, March 23, 2018.

Turk LS, Kuang X, Patel K, Dai W, Comoletti D. Structural Characterization of Reelin Using Cryo-Electron Tomography. Biotechnology Training Program Annual Mini-Symposium, Rutgers University, New Brunswick, June 25, 2018.

AWARDS

Rutgers Graduate School of Biomedical Sciences Excellence Award 2016-2017
Rutgers Institute for Quantitative Biomedicine Joint PhD Excellence Award 2016-2017

RAHUL UPADHYA
Advisor: Adam Gormley

Cancer is a major clinical concern in the U.S., as there are 1.7 million new cases each year. Despite massive investments into developing novel cancer therapeutics, many clinical treatment approaches are non-specific (e.g. chemotherapy and radiation therapy) with limiting toxicities. The pharmaceutical industry is shifting towards developing protein-based drugs that display improved tumor specificity. However, obstacles include high cost, immunogenicity, and aggregation in situ. Furthermore, proteins are particularly sensitive to changes in pH, temperature, and other solution conditions. This creates challenges when formulating biologics. Protein manufacturing is a major challenge because of instability, storage issues, and other complexities of manufacturing.

Our lab aims to develop systems of soft material polymer-peptide conjugates that trigger a proportional biological response to proteins. These polymer-peptide conjugates would resolve the major issues of protein manufacturing – instability, immunogenicity, and off-target toxicity. Polymers have enhanced solution stability, making storage concerns nonexistent.

PET-RAFT, a polymerization technique co-discovered by Prof. Adam Gormley, enables efficient polymerizations in 96- or 384-well plates. This represents a vast improvement over traditional polymerization techniques as PET-RAFT is oxygen-tolerant. Because of its oxygen-tolerant nature, we can design vast polymer libraries and easily vary parameters of chain length, monomer composition, and peptide incorporation. With the means to edit the polymer blueprint, we can design polymers with charge and backbone characteristics that avoid the problem of protein aggregation.

Our near-term objective is to rationally design polymer-peptide conjugates for therapeutic applications, specifically by incorporating a peptide of TNF-related apoptosis-inducing ligand (TRAIL) that induces apoptosis in cancer cells. This project has 3 components: (1) synthesis of polymer-peptide conjugates; (2) extensive characterization to understand structure; (3) biological screening and ligand validation. We hypothesize that altering the polymer design parameters will enable us to create a wide variety of polymer-peptide structures, some of which will optimally display the therapeutic peptide and consequently trigger biological activity. Therefore, through this characterization and rational design of polymer-peptide conjugates, we can recognize structure-activity relationships specific to cancer therapies.

In order to achieve this goal, extensive synthesis and characterization will be carried out. In the short-term, I will be utilizing resources for synthesis and size characterization. This includes size-exclusion chromatography (SEC), multi-angle light scattering (MALS), and dynamic light scattering (DLS) to determine molecular weight, polydispersity, and hydrodynamic size. I have already begun carrying out small-angle X-ray scattering (SAXS) experiments and will continue to do so in the future at the National Synchrotron Light Source II (NSLS-II). SAXS provides quantification of size, compactness, flexibility, and folding behavior. In the long term, I will be incorporating other characterization techniques to fully relate design parameters and structural consequences. Fluorescence techniques such as Förster resonance energy transfer (FRET) will verify polymer compactness, while small-angle neutron scattering (SANS) can quantify peptide display to maximize receptor interaction and surface plasmon resonance (SPR) can validate polymer-peptide conjugate binding to receptors. The overall goal is to fully characterize the structure of various polymer-peptide conjugates in order to establish a structure-activity relationship between the TRAIL polymer-peptide conjugates and cancer cell apoptosis.

PRESENTATIONS

Kosuri S, **Upadhya R,** Tamasi M, Gormley AJ. Combinatorial and High Throughput Testing of Protein Interfacing Polymer-Peptide Conjugates. Controlled Release Society Annual Meeting & Exposition, July 2018.

Kosuri S, **Upadhya R**, Tamasi M, Gormley AJ. Combinatorial High Throughput Testing of Polymer-Peptide Conjugates for Death Receptor Mediated Apoptosis in Cancer. CINJ Retreat, May 2018.

AWARDS

Rutgers University Excellence Award 2017-2018

CAROLINE WOOD
Advisor: Jay Sy

The Sy lab aims to develop materials, devices, and strategies to more effectively treat brain disorders and injuries. Delivering therapies to the brain is a challenging obstacle due to the delicate nature of the tissue, the presence of the blood-brain barrier, and the quick clearance rates of the drugs that have successfully penetrated it. The research I worked on this past year aims to test the hypothesis that drug exposure in the brain is reduced due to clearance via cerebral spinal fluid (CSF) efflux mechanisms. We aim to study drug clearance mechanisms and improve the treatment efficacy of glioblastoma therapies by controlling CSF dynamics. To accomplish this, I have been comparing brain epithelial cells sources to optimize an *in vitro* model of the choroid plexus and assisting in rodent studies that evaluate the effects of potential CSF modulating drugs. To develop the *in vitro* model of the choroid plexus, the tissue responsible for actively secreting CSF, I have compared the Z310 cell line and primary cells isolated from rat brains. For the primary cells, we are using techniques such as magnetic-activated cell sorting (MACS). Western Blot, immunocytochemistry, and flow cytometry have process and analyze cell purity. Cells are currently being cultured on Transwell membranes which allows us to study the effects of various drugs before introducing them to the animal model. Selection of the optimal cell source will allow us to produce an *in vitro* model that is most representative of the *in vivo* microenvironment. In addition, I have assisted in *in vivo* studies that have demonstrated the effects of two drugs, acetazolamide and verapamil, in decreasing and increasing the production rates of CSF, respectively. The drugs were injected intraventricularly in rat models with a fluorescent tracking dye that enabled us to quantify tracer clearance from the ventricles over time. I have also begun synthesizing biodegradable polyanhydride copolymers, which we plan to use in future animal studies with identified CSF modulators to show how the distribution and efficacy of currently used glioblastoma treatments will be affected by reducing CSF efflux.

PRESENTATIONS

Wood C, Browe D. et al. Optimization of electroactive hydrogel characteristics for use in a composite skeletal muscle scaffold. Biomedical Engineering Conference, Minneapolis, MN, October 2016.

Wood C, Figueroa M. Analytical method to fabricate reproducible SERS substrates. 252nd American Chemical Society National Meeting, Philadelphia, PA, August 2016.

Wood C, Biernacki L, DiMartini E, Magnotta A. A Dedicated Venous Stent for May-Thurner Syndrome. 2017 Northeast Bioengineering Conference, Newark, NJ, April 2017.

AWARDS

Eugene V. Dubois Graduate Fellow 2017-2018

Papers Published

Acevedo A, DuBois DC, Almon RR, Jusko WJ, Androulakis IP. Tissue-specific Circadian Regulation of Metabolic and Signaling Pathways in Multiple Tissues. Physiological Genomics. 2019. Submitted.

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APPENDIX D: RUTGERS UNIVERSITY BIOTECHNOLOGY TRAINING PROGRAM
INDUSTRIAL ROTATIONS
SUMMER 2018

STUDENT	DEPT/ADVISOR	COMPANY	PROJECT
Larry Cheng	Serom Lee	Defined Health	Indication Prioritization of Product X; Opportunity Assessment of Product Y; Competitive Landscape Assessment of Product Z
Josh Leipheimer	Jin Park and Dan Olsen	MDI	Assembly design and build of a single-wire pattern braided aortic stent made from nitinol metal.
Jeffrey Luo	Jumming Yie	Merck	Characterization of different luciferase species in functional cell-based assay.
Jenna Newman	James Li	Celularity	Utilizing CRISPR/Cas9 to delete genes implicated in immune evasion in Chimeric Antigen Receptor (CAR) T cells
Victor Tan	Agnes Yeboah and Renea Faulknor	Celgene	Regulatory in Chemistry, Manufacturing, and Controls Department, emphasis on Biologics
Liam Turk	Jiajun Mei and Shirley Ruan	Aleon Pharma	Regulatory Affairs – Investigational New Drug Application (IND) for an Anti-Pd1 Monoclonal Antibody (mAb)

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STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Jeffrey Luo

Department and Advisor: Chemistry and Chemical Biology, Dr. KiBum Lee

Corporation: Merck

Mentor: Dr. Junming Yie

Project Title: Characterization of different luciferase species in functional cell-based assay.

General Objective of the Project: The objective is to characterize the response of various luciferase variants for cell-based biological potency assays.

Student's Contribution to the Project: Important contributions include execution of transfection and cell assays, analysis of dose-response curves for model biological drugs, optimization of assay conditions, and issuing recommendations for future assay development efforts.

Techniques Learned: Notable techniques learned include non-viral transfection, general luciferase expression assays, and data acquisition and documentation in-line with Good Laboratory Practices

Student Comments on the Company and Mentor: The inclusion of an internship mentor (specializing in navigating industrial workplace) in addition to a project mentor (expert in the particular research field) is a very good idea. Aforementioned mentor is a project mentor who was quite understanding of the limitations of the internship and pleasant to work with.

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STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Larry Cheng

Department and Advisor: Quantitative Biomedicine; Bin Tian

Corporation: Defined Health

Mentor: Serom Lee

Project Title:

- Indication Prioritization of Product X
- Opportunity Assessment of Product Y
- Competitive Landscape Assessment of Product Z

General Objective of the Project:

- Recommend to the client an indication for Product X to proceed into clinical development
- Evaluate the opportunity of Product Y in an indication for the client
- Survey the strengths and weaknesses of current and potential competition of Product Z for the client

Student's Contribution to the Project:

- Evaluating and extracting information from publicly available sources including scientific literature, company press releases, analyst reports, and patents
- Participated in interviews with key opinion leaders
- Coalescing information into PowerPoint slides that will be presented to the client

Techniques Learned:

- Crafting effective PowerPoint presentation slides
- Searching and interpreting patent documents
- Exposure to conducting interviews with KOLs
- Construct a target product profile for a biopharmaceutical

Student Comments on the Company and Mentor:

- The mentor and the entire company fostered an excellent environment to learn about the consulting world, especially with a life science PhD background.
- Would recommend to future fellows to complete the Innovation & Entrepreneurship class as well as the Bioengineering in the Biotechnology and Pharmaceutical Industries course before participating in this internship.

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STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Josh Leipheimer

Department and Advisor: BME. Dr. Yarmush

Corporation: Medical Device Imagineering (MDI)

Mentor: Jin Park and Dan Olsen

Project Title: Assembly design and build of a single-wire pattern braided aortic stent made from nitinol metal.

General Objective of the Project: My project was involved in the build-design and assembly of a custom-made aortic stent made exclusively from nitinol metal. Nitinol is a biocompatible, superelastic and shape memory alloy, making it ideal for medical stent applications requiring high elastic yield. My work consisted of creating the assembly instructions for manufacturing a single-wire braided aortic stent made from nitinol metal, rather than traditional stain-less steel. The final result of my project was a successfully assembled aortic nitinol stent for veterinary applications.

Student's Contribution to the Project: My contribution to the project was involved with creating the initial assembly design instructions to successfully create a single-wire nitinol braided aortic stent. My other duties involved: Instron radial and tensile strength testing of medical stent implants, device calibrations, and quality control related tasks involved with maintaining equipment.

Techniques Learned:

- Mechanical testing of various aortic stents using Instron machine to meet FDA regulations and standards. (Radial and tensile testing in a temperature-controlled environment).
- Electropolishing techniques.
- Laser cutting equipment usage for stent manufacturing.
- CAD designing for 3D printing.
- Stent characterization using imaging techniques and mechanical testing.

Student Comments on the Company and Mentor: The industrial experience I gained at MDI gave me a fresh perspective on how the medical device industry in stents operates and functions. I not only learned new technical skills such as Instron mechanical testing to meet FDA standards, but also professional skills involved with the management and business side of medical device development. Jin Park and Dan Olsen were fantastic mentors dedicated to exposing me to both the technical and business aspects of stent development.

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STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Jenna Newman

Department and Advisor: Genetically Modified T Cells, Dr. James Li

Corporation: Celularity, Inc.

Mentor: Dr. James Li

Project Title: Utilizing CRISPR/Cas9 to delete genes implicated in immune evasion in Chimeric Antigen Receptor (CAR) T cells

General Objective of the Project: The Genetically Modified T Cell Group at Celularity, Inc. seeks to employ the latest cutting-edge gene-editing techniques to modify T cells to become enhanced mediators of tumor cell death.

Student's Contribution to the Project: My objective this summer was to use CRISPR/Cas9 to delete genes in CAR-T cells that could be hindering anti-tumor directed cytoxicity mediated by CAR-T cells. I developed processes for successful knockdown of genes of interest in primary T cell lines, and tested functionality of these tumor-killing T cells by cytokine assays and co-culture cytotoxicity assays.

Techniques Learned: CRISPR/Cas9, cytokine assays, cytotoxicity assay

Student Comments on the Company and Mentor: Celularity is a great environment to work in; everyone is very collaborative and friendly, and its small size lends itself to these attributes very well. I learned a lot in group lab meetings—and company-wide gatherings—on a variety of different research topics, which was very enlightening. My mentor, Dr. James Li, provided me with a great set of tools to acclimate to the CAR-T cell field, and gave me advice and support through this process. He was also flexible with respect to the projects that I wished to work on—which was beneficial for my learning experience at Celularity. I had a great summer internship at Celularity and am very thankful for this experience!

Biotechnology Training Program
Biomedical Engineering Building
Rutgers, The State University of New Jersey
599 Taylor Road
Piscataway, NJ 08854

Biomedical.rutgers.edu/biotech
Phone: 732-445-4500 x6284
Fax: 732-445-3155
Email: empresa@rci.rutgers.edu

STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Victor Tan

Department and Advisor: Pharmacology, Shengkan Jin

Corporation: Celgene

Mentor: Agnes Yeboah, Renea Faulknor

Project Title: Regulatory in Chemistry, Manufacturing, and Controls Department, emphasis on Biologics.

General Objective of the Project: Celgene has several products in the pipeline which require regulatory approval from government agencies before commercialization can happen. The project involves learning about the necessary regulation on biologics specifically and how to author the specific documents with regards to the quality of the product. Proper filing of regulatory paperwork results in approval of drug use in patients.

Student's Contribution to the Project: Supported authoring of necessary dossiers for regulatory filing for commercialization of biologic products.

Techniques Learned: Authoring BLA, IND, IMPD, NDA dossiers for regulatory filing.

Student Comments on the Company and Mentor: While the company is considered large by regular standards, there are still many areas that need improvement. There will be growing pains when learning certain things in the pharmaceutical industry, especially so given the advent of biologics as drugs. I've certainly learned a great deal from my mentor on how to approach each problem in a flexible manner. It is important to remember that ultimately, the product is meant for the patients

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STUDENT INTERNSHIP REPORT SUMMER 2018

Student Name: Liam Turk

Department and Advisor: Davide Comoletti – Biochemistry/Neuroscience & Cell Biology

Corporation: Aleon Pharma International Inc.

Mentor: Jiajun Mei, PhD and Shirley Ruan

Project Title: Regulatory Affairs - Investigational New Drug Application (IND) for an Anti-Pd1 Monoclonal Antibody (mAb)

General Objective of the Project: To compile, produce and submit and IND to aid a client in the FDA approval process for an anti-PD1 mAb for use in specific cancers.

Student's Contribution to the Project: Reviewing documents (such as experimental procedures and data regarding the mAb), creating reports, and delivering presentations on the scientific theory with respect to protein/mAb purification and production.

Techniques Learned:

- Preparing an IND
- Reviewing preclinical data and applying it in the proper context of an IND
- Developing a familiarity with the various modules within new drug applications
- Scientific writing skills to clearly convey the results in experimental data
- Analytical skills in identifying important experimental data points to include in experimental summary sections of an IND.

Student Comments on the Company and Mentor: The mentors and the company both provided a comfortable and amiable environment to work efficiently. Both Jiajun and Shirley were knowledgeable and approachable when help was needed, as I was completely inexperienced within the field of regulatory affairs prior to the internship. I think it would have been more helpful if there was a more structured training period during which the intern could become more familiar with the work and process of FDA drug approval, however I don't know if it is possible given the small size of the company. The small size did provide a "family feel" to the work environment and everyone got along well with each other. The CEO, Andrew Jiang, too was very approachable and mindful when it comes to the experiences of the interns. Interns, of which there were three during my time there, were expected to give biweekly presentations as a means of staying on track with work and also provided important practice and feedback in regard to work-related presentations.

16:125:603 Topics in Advanced Biotechnology

Fall 2018
Fridays, 9:00-11:00, BME Room 116

DATE	TOPIC	FACULTY MEMBER	FELLOW PRESENTER	FELLOW PRESENTER
September 7	Introduction: Getting the Most Out of Graduate School	Martin Yarmush	Mollie Davis	Josh Leipheimer
September 21	iJOBS	Susan Engelhardt	Larry Cheng	Emily D'Martini
October 5	Mentorship	Ann Stock, Xenia Morin, Evelyn Erenrich	-----	Anton Omelchenko
October 19	**BMES ANNUAL CONFERENCE (NO SESSION)**			
November 2	Ethics	Troy Shinbrot	Yoliem S. Miranda Alarcon	Jeff Luo
November 16	Academia Perspective	Maribel Vasquez	Victor Tan	Isabel Perez
November 30	Industry Perspective	Serom Lee	Liam Turk	Brandon Newton

- Faculty and alumni presentations are limited to **30-minutes** leaving 10-15 minutes for discussion, unless otherwise specified above
- Fellow presentations are limited to **10 slides**, and no more than **15 minutes** to allow for adequate discussion and feedback

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Design of a Hand-held Automated Venipuncture Device for Blood Draw and IV Catheter Insertions.

Josh Leipheimer
Yarmush Lab

Friday, September 7th, 2018
Rutgers University, Department of Biomedical Engineering

Venipuncture

The most common medical procedure

1.2 billion blood draws per year in the U.S.

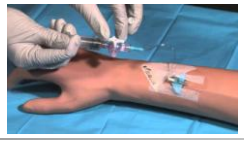
90% of hospitalized patients had some form of vascular access[1,2]

1. CDC National Hospital Ambulatory Medical Care Survey: 2015. 1–31 (2015).
2. McCann M, et al. *Journal of Nursing and Emergency Care* 34, 77–84 (2008).

Venipuncture | Venous Access

Venipuncture : The process of obtaining intravenous access for either:

Blood retrieval



Intravenous therapy

Venipuncture | Problems Close Up

Venipuncture failure: Missed first-stick, bruising, bleeding, inadequate blood sample, accidental needle sticks.

Pediatric Cases[7]

- Of the 249 pediatric patients:
  - 47% first-attempt failure.
  - Average 2.9 attempts to gain peripheral venous access.

Adult Cases[8]

- Venipuncture failure rates:
  - 14% for non-DVA patients
  - 27% for non-visible veins
  - 40% for non-palpable
  - 60% for emaciated

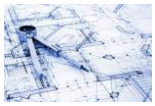
7. Lininger, RA 2003, 'Pediatric peripheral IV insertion success rates', Pediatric Nursing, vol. 29, no. 5, pp. 351-354.
8. Carr. "Insertion of peripheral intravenous cannula in the Emergency Department: factors associated with first-time insertion success", The Journal of Vascular Access. 2015; vol. 17, no. 2, pp. 182-190

Venipuncture | Our Goal

Our goal is to create a venipuncture device that can **safely**, **quickly**, and **efficiently** perform venipuncture procedures on patient's with difficult venous access (DVA).
- Routine blood draws
- IV catheter insertions

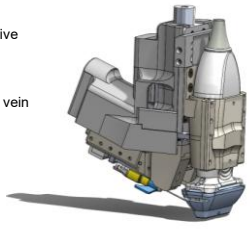
Areas of application:
- Hospital: bed side, phlebotomy cart
- Ambulance / emergency room setting
- Military, field of action
- Laboratory: blood draw and analysis

For a device such as this to be clinically viable:
- Small, **portable**, and easily maneuverable.
- **Easy to use** / require little to no training.
- **Efficient** and quick
- Economically viable: **cheap**.

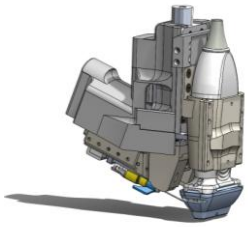


Gen5 Automated Venipuncture| Passive Arm Device

- 3 DOF:
  - 1) **Injection Motor:** Spindle drive translation mechanism.
  - 2) **Z-axis Motor:** Linear spindle drive stage. Raise and lower.
  - 3) **Needle Alignment:** -Y-axis
- Out-of-plane ultrasound used to image vein depth and alignment with needle.
- Force sensor for detection of needle puncture.
- Weight: 1.1 lbs.
- Attaches to movable arm for stability.



Gen5 Automated Venipuncture| Passive Arm Device



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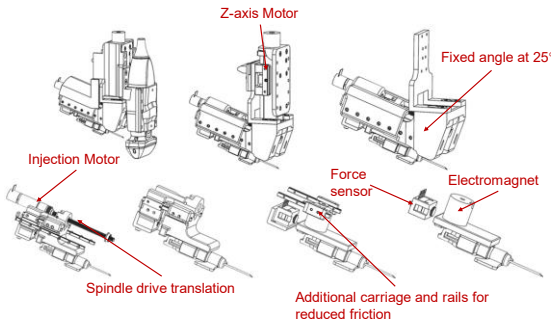
Gen5 Automated Venipuncture| Passive Arm Device



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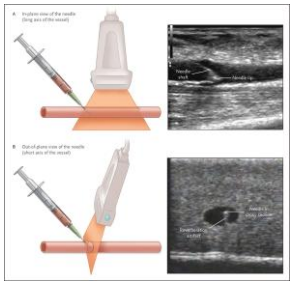
Hand-held Venipuncture| Ultrasound

- In-plane Ultrasound:
  - Transverse view
  - Difficult to image small needles and veins.
  - Needle and vein must be perfectly aligned with ultrasound image plane.
- Out-of-plane Ultrasound:
  - Cross-sectional view of needle and vein
  - Easier to identify needle and suitable vein.
  - Needle does not have to be directly in line with ultrasound plane.



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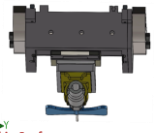
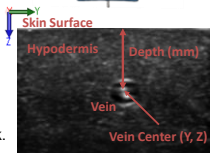
Hand-held Venipuncture| Alignment DOF

- Ultrasound probe must be centered directly above vein for insertion.
  - Once needle and vein are centered, device will signal to user that insertion can begin.
- **Problem:**
  - Small movements from patient and/or clinician can cause needle to **mis-align**.
  - Manually centering the ultrasound perfectly over the target vein is difficult and increases the risk of a missed stick.





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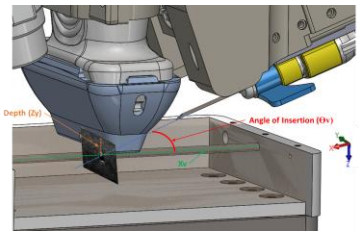
Gen5 HAVD| Kinematics and Ultrasound



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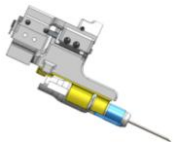
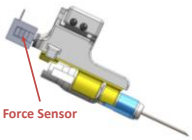
Hand-held Venipuncture| Force Sensing at Needle Tip

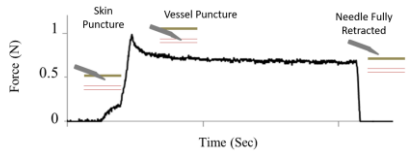




- During traditional venipuncture, clinicians can haptically “feel” when the needle has punctured the vein wall.
- Can we capture this “feeling” of the vein puncture using a force sensor in line with the needle during insertion?
  - Is it possible to determine vein puncture solely from the insertion force profile?
- Develop a passive device that assists clinicians in venipunctures by stopping needle progression when puncture is detected.

12

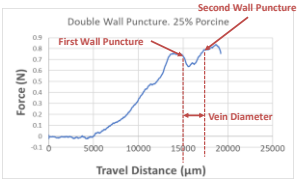
Hand-held Venipuncture| Force Sensing at Needle Tip



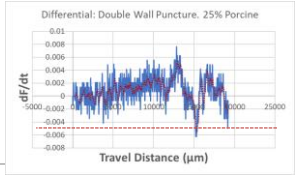
- For puncture detection, we need to observe not the amplitude of force, but the **change in force**, or the differential of the force signal.
- A large drop in force is associated with the needle puncture the first initial vein wall.
- Any large drop in force will result in a large negative differential spike.
- What is the best negative differential **cutoff value** for puncture detection across **all** demographics?

Hand-held Venipuncture| Force Sensing at Needle Tip

- Preliminary results from force sensor testing on phantom arm.
  - Vein diameter = 3 mm
  - Vein Depth = 6 mm
- Double vein wall puncture test.



- Blue is unfiltered
- Red dotted line is moving average filtered signal (M = 5)
- From this single result, cutoff value of **-.005** would give puncture detection.



Hand-held Venipuncture| What's Next

What's Next:

1) Clinical trials using Gen5 semi-hand-held device.
   - Data to obtain: force profiles, motor positions, ultrasound images, motor current, time to completion, successful sticks, etc.

2) Investigate predication and machine learning algorithms for determining successful needle sticks from large amounts of vein puncture force profiles and US imaging.
   - Bayesian / structured prediction.
   - Use animal, arm cadaver models for accurate model device tests.

3) Develop a custom **Inertial Navigation System** (INS) that also utilizes **force sensor data**, **ultrasound imaging**, and **secondary camera imaging** to determine the pose of a free-floating, hand-held venipuncture device.
   - Allow device to become truly portable.
   - IMU's: gyroscopes, accelerometers, and magnetometers.

Team | Rutgers University

- Max Balter, PhD
- Alvin Chen, PhD
- Anil Shrirao, PhD
- Zach Lopez, undergrad
- Nicholas Demaio, undergrad
- Hill Chang, undergrad
- Martin Yarmush, MD, PhD

National Science Foundation · NATIONAL INSTRUMENTS · XILINX · NIH National Institutes of Health · IEEE SPECTRUM · vasculogic

Questions or comments?

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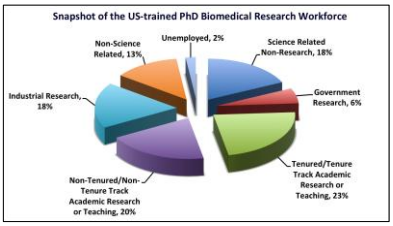
Rutgers iJOBS

September 2018

Changing Times

According to a recent report by the NIH Biomedical Workforce Working Group, approximately
23% of PhD graduates will pursue academic positions,
while 77% will pursue other career options



Biomedical Research Workforce Working Group Report, NIH 2012

BEST Awardees

17 Universities
$2 million for 5 years
Infrastructure support
No direct fellow funding

1. Cornell U
2. Emory/Georgia Tech
3. NYU
4. U Mass Worcester
5. UC Davis
6. UCSF
7. U Colorado Denver
8. Vanderbilt U
9. Virginia Polytech
10. Wayne State

11. Rutgers U
12. U Chicago
13. U North Carolina
14. U Rochester
15. UC Irvine
16. Boston U
17. Michigan State

3

Excellent Professional Environment
The World's Cure Corridor
New Jersey

- Over 3000 life science and biopharmaceutical establishments
- 400 biotech companies
- 13 of the 20 largest biopharmaceutical companies globally
- 12 of the world's top medical technology companies
- #3 state for R&D investment, and bioscience-related patents
- 22,000 life sciences graduates annually

4

Professional and Trade Support Abounds

5

iJOBS Participation

RUTGERS

Rutgers Graduate Students
- SGS (New Brunswick/Piscataway/Newark)
- GSN (Newark)
- GSC (Camden)

Rutgers Postdocs
- RWJMS
- NJMS
- SAS New Brunswick
- SoE
- SoP
- SEBS
- SAS Newark
- SAS Camden

Free shuttle transportation provided

PRINCETON UNIVERSITY
STEVENS INSTITUTE OF TECHNOLOGY
NJIT
Rowan University

6

Experienced Program Leadership

Martin Yarmush	James Millonig	Janet Alder	Susan Engelhardt	Doreen Badheka
				
Distinguished Professor of Biomedical Engineering School of Engineering	**Senior Associate Dean** School of Graduate Studies	**Assistant Dean for Academic & Student Affairs** School of Graduate Studies	**Executive Director** Center for Innovative Ventures of Emerging Technologies	**Program Director** School of Graduate Studies
Co-PI	Co-PI	Co-PD	Co-PD	Co-PD

7

Phased Approach

Now that you are successful in your chosen career, share your experience with new trainees.

Ready yourself for career placement and search for a position.

Go forward and define your career focus. Apply for "trainee " status and, if accepted*, take advantage of individualized mentoring and career planning support.

Sample the program to see if a non-academic career is for you; register for programming as meets your needs.

8

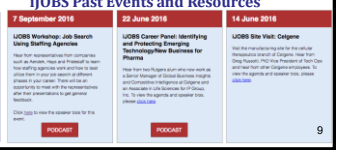
Communication Platforms

Email listserve: 1500 members

Trainee run blog: ijobs.rutgers.edu/blog.php

Website: ijobs.rutgers.edu

iJOBS Past Events and Resources



9

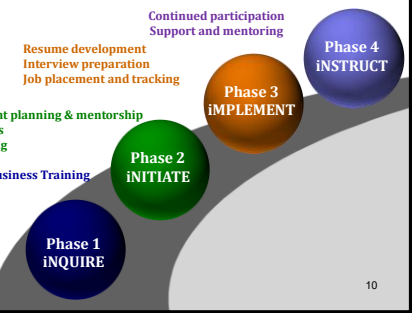
Holistic Programming

Continued participation
Support and mentoring

Resume development
Interview preparation
Job placement and tracking

Individual development planning & mentorship
Career-specific courses
Professional shadowing

SciPhD Leadership and Business Training
Site visits
Networking sessions
Career panels
Case studies
Primers
Workshops
Career fair
Web portal access



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Phase 1

11

Examples of Career Tracks

- Principal investigator
- Bench Research in Government, Pharma or Biotech
- Teaching-intensive careers in academia
- Science and Health Policy
- Patent Law
- Tech Transfer and Business Development
- Clinical Research
- Regulatory Affairs
- Health and Science Data Analysis
- Business Consulting
- Scientific Writing and Medical Communications
- Medical Affairs
- Non-profit and Foundations
- Finance and Equity Research
- Publishing
- Food safety
- Journalism
- Teaching Education Outreach
- Entrepreneur

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SciPhD: Leadership and Business Skills for Scientists

Provided by Human Workflows, LLC
Winter 2015, 2016, 2017, 2018/35 hours

- The Business of Science
- Major Leadership Styles
- Successful Communications as a Scientist
- Developing Your People
- Networking and the Interview Process
- Team Performance Tools
- Negotiations
- Financial Literacy
- Strategic Project Management for Scientists

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Representative Site Visits

- Merck (3)
- Genewiz
- Bristol-Myers Squibb (3)
- Novartis
- GlaxoSmithKline
- Regeneron
- Eli Lilly (2)
- Celgene
- Janssen
- Commercialization Center for Innovative Technology
- Enterprise Development Center
- Institute for Life Science Entrepreneurship
- Sanofi Aventis
- Covance
- Kashiv
- Ferring Pharmaceutical
- NJ Dept. of Health (3)
- Stryker
- Colgate-Palmolive
- Envigo

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Representative Workshops: Job Simulation





- Patent Law – rewrite patent to demonstrate originality
- Pharma Market Research Analysis – report on whether drug is worth pursuing
- Medical Affairs –Medical Informaticist vs MSL role play
- Consulting – recommend approach to launch new clinical trial
- Regulatory Writing – prepare an Investigative Brochure
- Medical Communications – create slide deck for physician
- Entrepreneur - How to test your biotech business idea

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Representative Workshops: Primers






- Project Management
- Python with DataCarpentry - programming and how to deal with large datasets
- Good Laboratory Practice (GLP)
- Pharmokinetics and Pharmodynamics (PK/PD)
- Immuno-oncology Research
- Communicating Science with Alan Alda - elevator pitch and improv to connect with audience
- Scientific Storytelling

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Representative Workshops: Job Search







- Targeted resumes
- LinkedIn profiles with photo shoot
- Networking skills
- Transferrable skills
- Interviewing skills
- Informational interviews
- Job search using staffing agencies
- Finding and applying for an internship
- Goal setting and time management
- How to prepare for job fairs
- International students seeking employment in USA
- Self assessments (StrengthsFinder, Birkman)
- Emotional Intelligence and influencing others

17

Networking Events

- All career panels, site visits and workshops have networking component since they are in person
- Coordinate with professional societies: Sino American Pharmaceutical Association, American Association of Pharmaceutical Scientists, and BioPharma Networking Group
- Rutgers and iJOBS alumni

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BioNJ/iJOBS Career Fair
April 2015, 2016, 2017, 2018
exclusive for life science companies

Trainee Opportunities through iJOBS Sponsorship

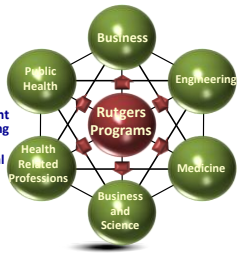
- Board Certification in Medical Affairs
- From Science to Pharma MSL Preparation
- What Can You Be with a PhD at NYU
- GRO Biopharma Conference with NY Schools
- BioNJ Inspiring Women in STEM
- Association for Women in Science
- Regeneron Science to Medicine Forum
- Biogen Drug Development Conference

Phase 2

Phase 2 iNITIATE

Career Track Example Skill Classes One 40-Hour Class

- Programming Methodologies for Numerical Computing
- Drug Discovery through Preclinical Development
- Introduction to Public Administration; Public Policy Formation
- Clinical Trials, Adverse Event Reporting, Post-Marketing
- Practical Aspects of Clinical Trial Design
- Bioengineering in Biotech and Pharma Industries
- Fundamentals of Regulatory Affairs



- Project Management; Perspectives in Drug Development
- US Healthcare System ad Pharma Managed Markets
- Drug Development: From Concept to Market
- Project Management
- Pharma Product Management
- Organizational Behavior
- Innovation and Entrepreneurship

Professional Shadowing and Mentoring

- Each trainee is matched to a mentor and a shadowing opportunity relevant to their chosen track with industrial, institutional or governmental partners. 72 hours over a whole semester or over 2 weeks.
- Each trainee is assigned a professional mentor and uses the Individual Development Plan (IDP) as a framework for growth.

We Thank Our Professional Shadowing Hosts

We Thank
Our Professional Mentors

Department of Food Science
SEDOR PHARMACEUTICALS
APOTHEKER GROUP THE FUTURE OF MEDICAL AFFAIRS
Reckitt Benckiser
Cymreg Consulting, LLC
FERRING PHARMACEUTICALS
SymBio
HEALTHLOGIX
CMPI
Celgene
RUTGERS New Jersey Medical
ETHICON
Allergan
Greenberg Traurig
MERCK
BD
NJHA
MediTech Media
accenture
Bristol-Myers Squibb
Huron CONSULTING GROUP
janssen
Logan Consulting
Milton Grannatt
PA Consulting Group

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Phase 3

Phase 3 iMPLEMENT

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Job Search Preparation

- One on one mentoring sessions with Juliet Chin Hart to refine resume and cover letter
- LinkedIn Counseling with 2Actify
- Strategize on job search approach
- Prepare for interviews

Actify™

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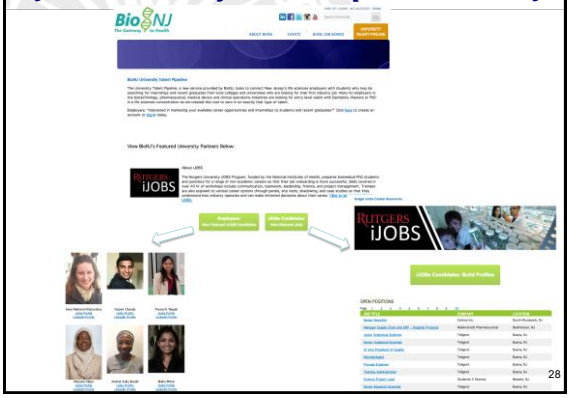
iJOBS University Talent Pipeline on BioNJ



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Outcomes

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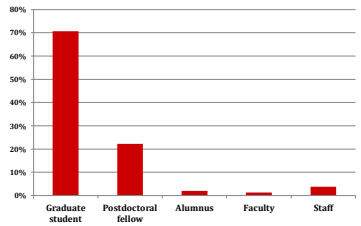
iJOBS Cohort Participation

In 4 years:
1790 eligible trainees
836 attended at least 1 event (46%)
16,258 person hours
111 non-Rutgers participants

Trainee Academic Standing



Total pool
71% predoc
29% postdoc

30

Overall SciPhD Outcomes

Within 3 years of program initiation, with a cohort of 153 highly engaged trainees, **62%** have completed either an iJOBS externship, independent internship and/or secured professional employment.

Years	Number of trainees	Phase 2 Externship %	Independent Internship %	Job or Postdoc %	Still in training %
2015-2017	153	28	13	41	59

31

Representative Jobs and Interships

Rob Connacher scientist at Merck. Fei Wang data scientist at Gartner Inc. Sarah Misenko GSK leadership program position. Dharm Patel position at Leo Pharma. Gangotri Dey Tech Transfer internship. *Peter Swiatkowski job at Celvive*. Nisha Mittal at Kashiv Pharma. Anna Hader postdoc at Johnson & Johnson. Almin Lalani co-op at Huron, internship at Novartis, postdoc at NCI. Eileen Oni Christine Mirzayan Fellow and AAAS Fellowship. Ina Nikolaeva Nucleus Global position. Kristina Hernandez MediTech Media position. **Winder Perez Stryker Corp Assistant Microbiologist.** Andrea Gray FDA/Center for Biologics Evaluation position. Myka Ababon Wiley Publishing copyeditor volunteers and job at Caudex Medical Writing Azadeh Jadali 3D Biotek position. Serom Lee consulting position at Defined Health. Harita Menon 3D Biotek position. Eva Rubio-Marrero Lilly internship and job at Celgene. Jimin Zhang Scientist position Insmed. Lisheng Zhou Genewiz internship and bioinformatist job at Mt. Sinai. Eva Rubio Marrero internship Eli Lilly and Job at Celgene. Chenchao Gao internship FDA Office of Clinical Pharmacology and job at Seattle Genetics. Yuan Liu Genentech internship. Jennifer Therkorn Rutgers Eagleton Institute of Politics Fellowship. Shanique Edwards Biology Science Writer at Draw it to Know it. Christina Ramirez internship at the Institut Pasteur Korea and Eli Lilly. Ileanna Marrero-Berrios internship at Celgene. Xiaowei Zhang internship at CSL Behring in the Clinical

32

Promotional initiatives

www.ijobs.rutgers.edu

33

"Rutgers iJOBS provides the opportunity to learn the business of science, some basics about industry, and skills that graduate students don't typically get in an academic program. It opens up people's eyes to being more professional and the power of networking. This program is something most students need, whether they are planning an academic career or they intend to work in industry or policy."

- Eileen Oni, PhD
Former PhD student at Rutgers
Currently AAAS Fellow at NSF

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"As a female graduate student in science, it was truly inspiring to be surrounded by successful women and men who work to push healthcare, science, and technologies in a direction that helps people all over the world. The event gave me a fresh perspective and reminded me that the work that I do as a scientist can truly have an impact on the quality of peoples' lives down the road."

- Paulina Krzyszczyk, PhD Candidate, Rutgers University
Speaking about the Women's Healthcare and Leadership Summit (hosted by the Healthcare Businesswomen's Association and sponsored by iJOBs)

35

"I absolutely enjoyed going to my shadowing sessions because with each experience I learn, I grow and I am inspired in my studies. I realize that some may think that doctoral students may be pulled away from their studies if they are not always in the lab. However that has not been my experience. As I watched the ways in which individuals solve a problem, I gained a better perspective on how to solve an issue that I may have bene experiencing. The opportunity to shadow has been powerful for me and I hope other students are afforded the same experience."

- Yaa Haber, PhD Candidate, Rutgers University

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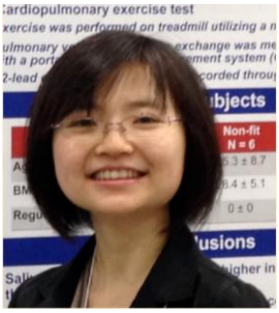
"I know that academic positions are hard to find these days, and grants are perhaps even harder to find. So what now?
I'm happy to say, the iJOBS program at Rutgers is helping me explore other options. From iJOBS events, I have started to broaden my horizons to include at least a rudimentary understanding of careers in industry, consulting, non-profit, and contract research organizations. Even if I stay the course and get an academic position, at least I'll go into it knowing the lay of the land."

- Bob O'Hagan, Post-Doc, Rutgers University



"Most of time I was observing their working, it was really help to see what they were doing and how they discussed with customers, coworkers, senior coworkers. I have learned the overall process of their work and soft skills from these observation. Because I have shadowed under different mentors, I was able to see the different aspects of medical communication company, not only helped me to think which department is good for me to start my career, but also let me have more options to develop my career as well in the future."

- Yang Chen, former doctoral student, Rutgers New Jersey Medical School, and currently an Account Executive at BioScience Communications at Edelman, Describing her shadowing experience at a medical writing company.

Questions?

www.ijobs.rutgers.edu

RUTGERS
THE STATE UNIVERSITY OF NEW JERSEY

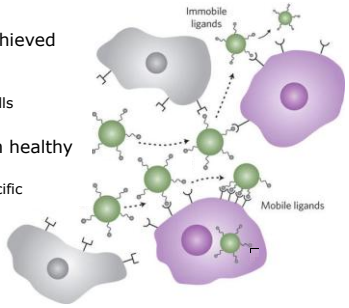
Free Radical-Mediated Immobilization of Therapeutic Factors

Emily DiMartini
Advisor: Dr. David Shreiber

September 21, 2018
Topics in Biotechnology I

Traditional Targeted Delivery

- Active targeting achieved by ligand-receptor interactions
  - Bind to diseased cells
  - Specific targeting
- Drugs cleared from healthy tissue
  - Not presenting specific receptor



Novel ligand-receptor interactions identified for each disease

Irvine. One nanoparticle, one kill. Nature. Slide 2 of 15

Free Radicals in Disease

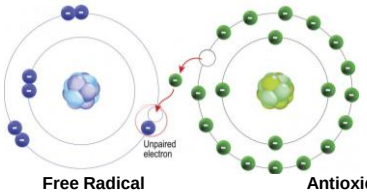
- Elevated free radical concentration is characteristic of various injury states
  - Highly reactive species with an unpaired electron
  - Localized to diseased tissue
  - Common across disease states

An elevated level of free radicals serves as an obstacle to healing

Slide 3 of 15

Free Radicals & Antioxidants

- Free radicals naturally occur in the body
  - Vital to cell signaling and other physiological processes
  - Reactive oxygen species (ROS) & reactive nitrogen species (RNS)
- Endogenous antioxidants consume the free electron
  - Attenuate reactivity
  - Natural mechanism to maintain normal free radical concentration

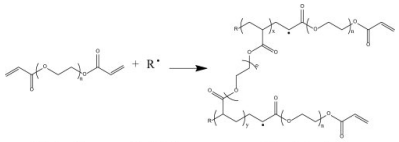


Free Radical **Antioxidant**

Free radicals overwhelm antioxidants in various disease states

Slide 4 of 15

Free Radicals in Biomaterials



PEGDA Free radical Cross-linked PEGDA

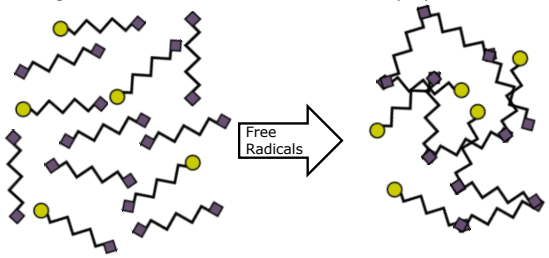
- Initiate crosslinking of acrylate groups on polymers
  - Induce rapid polymerization
- Polymerization terminated by radical-radical interactions
- PEGDA is normally crosslinked with a toxic photoinitiator

We propose that native free radicals can induce crosslinking of acrylated PEG. If a therapeutic was conjugated to the PEG, the delivery of that molecule would be targeted and sustained at the site of free radical production.

Slide 5 of 15

Proposed System

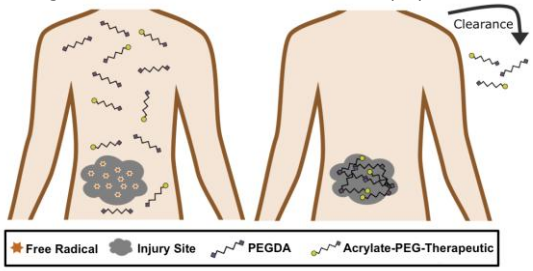
Drug immobilization via native free radical polymerization



Slide 6 of 15

RUTGERS Targeted Delivery

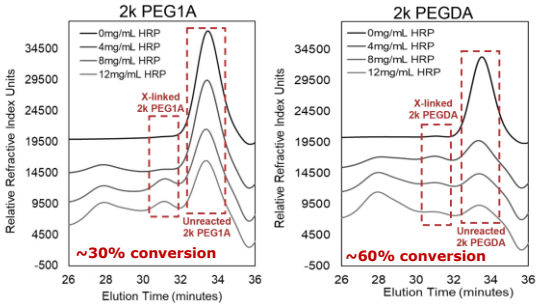
Drug immobilization via native free radical polymerization



Thus far, we have demonstrated that PEGDA reacts with free radicals encountered *in vivo*

Slide 6 of 15

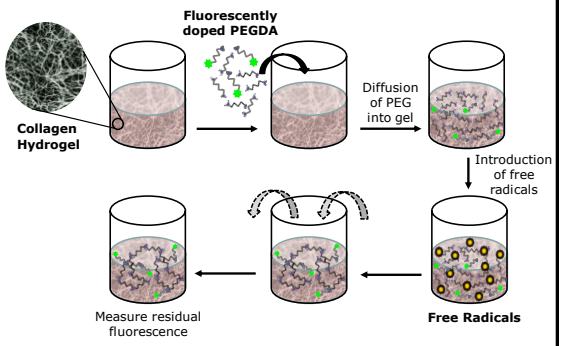
RUTGERS Gel Permeation Chromatography



ROS can effectively increase the size of acrylated PEGs

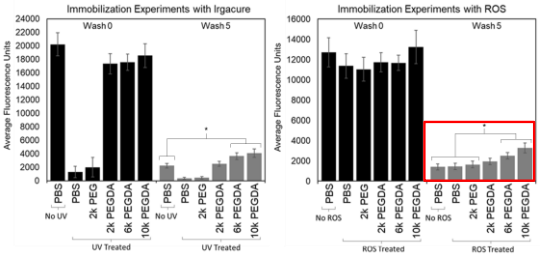
Slide 7 of 15

RUTGERS Immobilization Model



Slide 8 of 15

RUTGERS PEGDA Immobilization

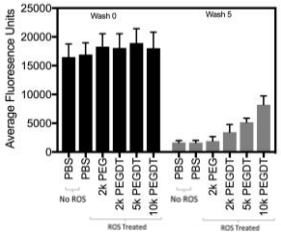


Significant increase in residual fluorescence demonstrates polymer immobilization

Slide 9 of 15

RUTGERS Potential Alternate Chemistries

Radical-initiated reactions: (1) thiol-thiol
(2) alkene-alkene
(3) thiol-alkene



Alternate chemistries may provide enhanced immobilization kinetics

Slide 10 of 15

RUTGERS "Clickable" Delivery

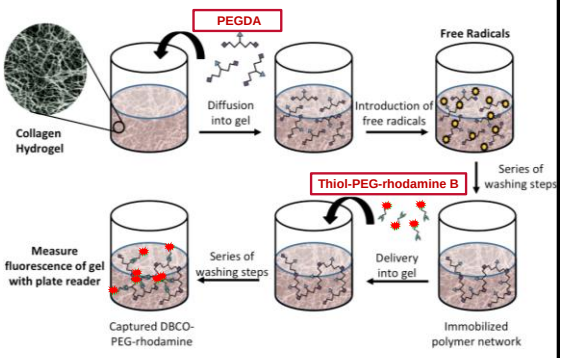
Delivering the therapeutic on the polymer backbone limits the crosslinking and formation of the polymer network

Proposed Second-Generation Model:
We are proposing a two-phase, two-component delivery system that localizes therapies for sustained presentation via click chemistry

Uncouple the system into two phases:
(1) Crosslinking reaction for polymer immobilization
(2) Drug delivery via chemistry

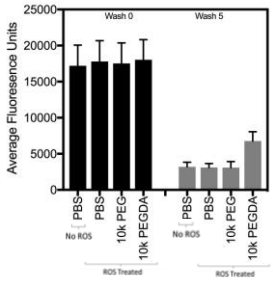
Slide 11 of 15

Proof-of-Concept Model



Preliminary Data

PEGDA network with subsequent Rhodamine B-PEG-Thiol delivery to leverage acrylate-thiol "click"



Proof-of-concept for two-phase "clickable" system

Future Studies

Future Directions:

- Characterization of alternate chemistries
- Development of "clickable" system

- Evaluating a therapeutic factor
- *In vivo* studies for drug localization and functional outcomes
  - TechAdvance Award
- Incorporate degradable linker for internalizing drugs

Acknowledgements

Advisor: Dr. David Shreiber
Chris Lowe
The Shreiber Lab

Funding Sources:
NIH Biotechnology Training Program (NIH T32 GM008339)
NJCBIR Pilot Research Grant (CBIR14FEL004)
TechAdvance (FP10535)

Alternative Polyadenylation in the Placenta

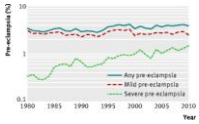
Larry Cheng

September 21, 2018

Topics in Advanced Biotechnology I

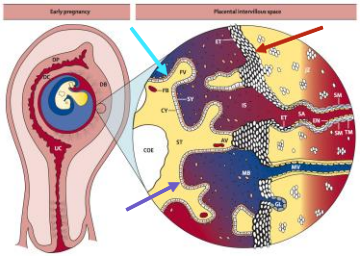
Placental Function is Important in Pregnancy and Offspring Health

- Vital for pregnancy
  - Chorionic gonadotropin (hCG) production
  - Nutrient/waste exchange
  - Immune tolerance
- Abnormalities contribute to:
  - Miscarriage: 15-20% ~ 1,000,000/year
  - Stillbirth: 0.5% ~ 24,000/year
  - Pre-eclampsia: ~2-8%
  - Fetal growth restriction: ~10%
- Potential impact on long term health
  - "Placental origins of adult disease"



Placentation Involves the Differentiation and Migration of Trophoblasts

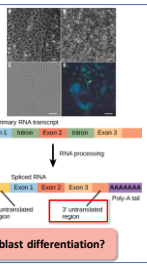
- **Syncytiotrophoblast (SCT)**
  - Multinuclear
  - Endocrine
  - Barrier
- **Cytotrophoblast (CT)**
  - Mononuclear
  - Germative
- **Extravillous trophoblast (EVT)**
  - Invasive
  - Anchor
  - Remodel



Commonly Used Trophoblast Models

- Primary trophoblasts from term placentas
- Trophoblast cell lines
  - Transformed
  - Choriocarcinoma
- Human embryonic stem cells (hESCs)-derived trophoblasts
  - BMP4 treatment to differentiate into trophectoderm lineage
  - Heterogeneous population

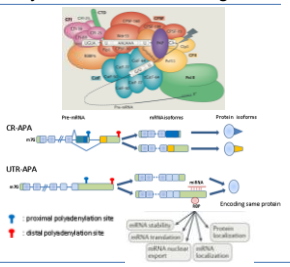
3'UTRs are shorter upon differentiation



What role does alternative polyadenylation play in trophoblast differentiation?

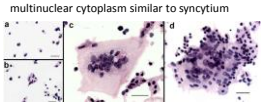
Alternative Polyadenylation (APA) is a Major Mechanism of Gene Regulation

- RNA-processing mechanism that generates distinct 3' termini
- Selection of polyadenylation site (PAS) is influenced by many factors
- Results in modification of gene expression
  - Protein isoforms (intron)
  - Distinct mRNA isoforms (3'UTR)
- Specific to tissue and environment
  - Brain
  - Tumor
  - Placenta?

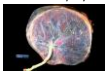


APA Analysis Using a Bulk RNA-Seq Dataset

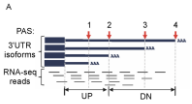
- hESC-derived trophoblasts
  - 40-70um and >70um fractions contain sheets of multinuclear cytoplasm similar to syncytium



- Purified cytotrophoblasts (CTs) from 3 placentas at term
  - *in vitro* differentiation into syncytiotrophoblasts (SCTs)



Significance Analysis of APA using RNA-Seq (SAAP-RS)

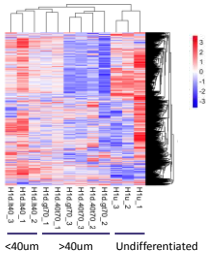


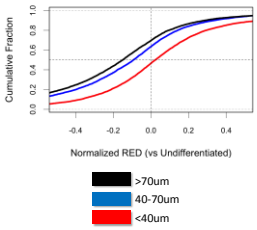
RED = ΔLog_2(DN/UP), test *vs.* reference

Large RED = **Longer** 3'UTR

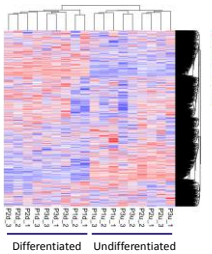
Small RED = **Shorter** 3'UTR

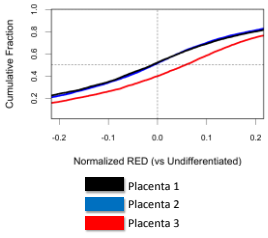
Cells in the Syncytial-Like Cell Sheets Have Shorter 3'UTRs





Two Out of Three Primary Samples Show Milder 3'UTR Shortening





Conclusion

- Summary
  - The placenta is a multifunctional organ that is important for fetal development
  - Alternative polyadenylation (APA) is one mechanism for gene regulation via production of different mRNA isoforms and/or protein isoforms
  - Trophoblast differentiation leads to global 3'UTR shortening
    - Preliminary data suggests 3'UTRs are shorter in syncytiotrophoblasts
- Future Work
  - Analysis of single-cell RNA-Seq data from primary trophoblasts
    - Functional analysis to identify biological processes affected by APA in trophoblast subtypes

Acknowledgements

- Tian Lab
  - Bin Tian (PI)
  - Dinghai Zheng
  - Qingbao Ding
  - Jihae Shin
  - Ruijia Wang
  - Aysegul Guvenek
  - Yiyang Zhao

NIH National Institute of General Medical Sciences

- Rutgers Biotechnology Training Program (T32GM008339)

RUTGERS

- Presidential Fellowship

Career Paths in Biomedical Engineering

Rutgers University
Department of Biomedical Engineering
November 2, 2018

Maribel Vazquez, Sc.D.
Associate Professor
Department of Biomedical Engineering

2018 The City College of New York

January 2019: THE STATE UNIVERSITY OF NEW JERSEY RUTGERS

OVERVIEW

*Experiences impact how we **choose** to develop careers*

Successful career paths aren't always straight lines



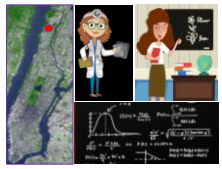
{Goals vs Opportunities} **OR** {Achievement vs Experience}
-Earl Nightingale

Rewarding careers can be build an assortment of both

- **My Background**
- **Undergraduate Years**
- **Industry Experiences**
- **Graduate School**
- **Faculty Career**
- **Research@Rutgers**

EARLY YEARS

My Background



- Native New Yorker
- Washington Heights (public schools)
- First US citizen in family (Caribbean)
- First to go to college (*Only to go away!)*
- First *(and only!)* engineer
- First PhD, researcher, faculty…..
- *Goals vs Opportunities?*

Mechanical and Aerospace Engineering

- New York State Scholarship
- First engineering internship: NASA
- Applied to PhD programs in Aerospace
- Loved Senior Design and Prototyping
- So I Covered bases with industry too

EMPLOYMENT

Industry Experiences

- Met industry rep at senior year engineering dinner
- Talked about design, manufacturing, microscale
- Started with a summer internship on '*West Coast*'
- 3 years--- 3 divisions--- 2 states (OR, CA)

Division I:
- General Engineering
- Water sources (RODI)
- AWN and EHOS
- Air filtration/delivery

Division II:
- Cleanroom Design
- New Construction
- Micro-contamination
- Process support

Division III:
- Microfabrication
- Photolithography
- 4in-9in process

GRADUATE SCHOOL

Transition to Graduate School

- Industry provided integrated training in engineering - - - So Exciting!
- Production was division(s) goal and I wanted technology application
- Employment revealed a few real-world truths:
  1. Not everybody is a good boss! Bad ones make life hard.
  2. Need balance between doing what you **want-** and **have-** to do
  3. Increased education offers greater project flexibility
- *Achievements vs Experience?*
- Corporate mechanisms paid for my graduate school and kept my benefits and salary on hold for 9 academic months each year.

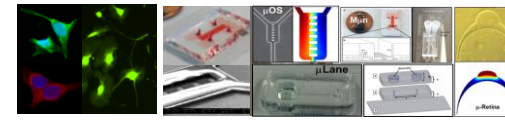
Mechanical Engineering (MS, PHD)

- Focused biotechnology research
- Collaborations with biologists and other scientists
- Impacts of educational training on under/graduate students

FACULTY ROLES

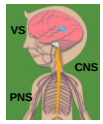
Biomedical Engineering: My Home Department

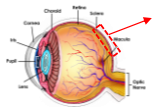
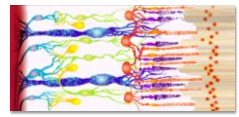
- Co-founder of new BME Department (est. 2002)
- Minority Serving Institution (MSI, >51% URM)
- Non-Traditional Students (>78%)
  - ✓ First-Gen/ First-in-College
  - ✓ 2nd Degree/Older
  - ✓ Work>30hrs/week
  - ✓ Veterans/Disabled



- **Microfluidics** create controlled extracellular environments to study cell behavior
- Devices provide a variety of external stimuli including pressure, flow, diffusion, electric fields, mixing and combinations thereof

GLIA & THEIR PRECURSORS

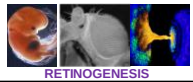


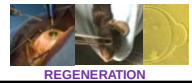



- The **RETINA** enables vision via conversion of photonic energy into sight
- Retinal tissue contains >100 Billion cells (7 lineages) across 500μm
- Signaling that guides eye development is well-conserved across species
- Stem cell-based therapies recapitulate development for regeneration
- Microdevices enable study of Cell Fate, Behavior and Connectivity needed

New Home @Rutgers BME

- Collaborations with other BME researchers and mentoring of doctoral students


RETINOGENESIS


REGENERATION

CLOSING POINTS

- *Success is the top of the mountain and you **WILL** get there*
- *Career paths **do not** have to connect in straight lines*
- ***Choose** enriching opportunities, experiences and achievements*

THANK YOU FOR YOUR ATTENTION!


STEM CELL DIFFERENTIATION CUE LOCALIZATION VIA NANOMATERIAL-COATINGS FOR REGENERATIVE THERAPIES

2018-11-02

Jeffrey Luo

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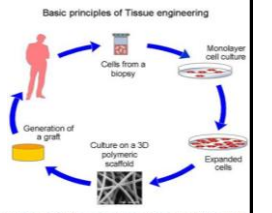
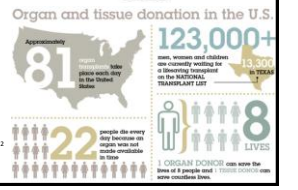
What is Tissue Engineering, and Why?

- Field of **augmenting, replacing, or supplementing** human body parts
- Demand arising from transplant **supply/demand mismatch**
  - Diseases, injuries, genetic predisposition
- **Rectify side effects & deficiencies**
  - Requires lifelong immunosuppressant use
  - Chronic rejection leads to inevitable organ dysfunction/failure
  - Can help patients with unusual morphologies

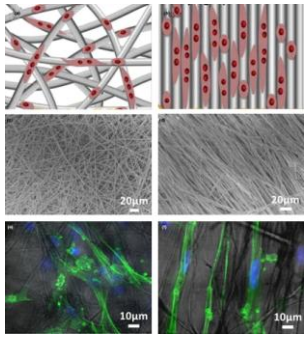




http://textile.iitd.ac.in/highlight/foil8/image002.jpg
https://www.donatelifetexas.org/wp-content/uploads/2015/07/LIF-OrganAndTissueDonationInUS-7.21.15.jpg

Nanofibers vs Bulk Hydrogel

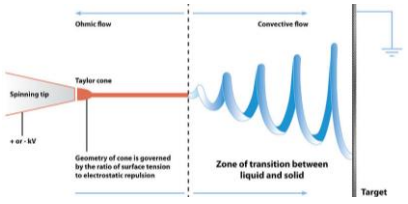
- 1-100 nm diameter fibers
- Mimic extracellular matrix fibers and to align cells
  - Blood vessels, muscle fibers, neuronal axon mimic
- Advantages of nanotopography in tissue engineering
  - ***High surface-to-volume ratio***
  - ***High diffusivity/porosity***
  - ***Cell alignment***
  - ***Anisotropic mechanical reinforcement***



Wang, Xianfeng, Bin Ding, and Bingyun Li. "Biomimetic electrospun nanofibrous structures for tissue engineering." *Materials Today* 16.6 (2013): 229-241.
Manoukian, Ohan S., et al. "Electrospun Nanofiber Scaffolds and Their Hydrogel Composites for the Engineering and Regeneration of Soft Tissues." *Biomedical Nanotechnology: Methods and Protocols* (2017): 261-278.

Electrospinning

- Use of electric force on a solution to create fibers
  - Electrostatic repulsion overcomes surface tension and removes solvent



- Problems with loading growth factors (proteins)
  - **Volatile organic solvents, high electrical potential, dehydration, shearing**

Garg, Koyal, and Gary L. Bowlin. "Electrospinning jets and nanofibrous structures." *Biomicrofluidics* 5.1 (2011): 013403.
Joanna Gatford, The New Zealand Institute for Plant and Food Research Ltd

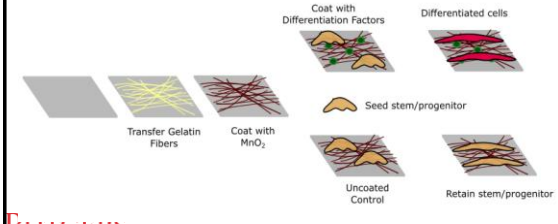
Nanomaterials (GO and MnO_2)

Property	Graphene Oxide (GO)	Graphene	Manganese dioxide (MnO_2)
Electrical conductivity			
Hydrophilicity			
Biodegradable	(?)	(?)	
Cytotoxic			
Protein/molecule Absorption			

Lvov, Yuri, et al. "Films of manganese oxide nanoparticles with polycations or myoglobin from alternate-layer adsorption." *Langmuir* 16.23 (2000): 8850-8857.
Liu, Jinbin, et al. "Toward a universal "adhesive nanosheet" for the assembly of multiple nanoparticles based on a protein-induced reduction/decoration of graphene oxide." *Journal of the American Chemical Society* 132.21 (2010): 7279-7281.
Qian, Yong, Shunbao Lu, and Fenglei Gao. "Synthesis of manganese dioxide/reduced graphene oxide composites with excellent electrocatalytic activity toward reduction of oxygen." *Materials letters* 65.1 (2011): 56-58.
Guo, Xiaoqing, and Nan Mei. "Assessment of the toxic potential of graphene family nanomaterials." *Journal of food and drug analysis* 22.1 (2014): 105-115.
Deng, Renren, et al. "Intracellular glutathione detection using MnO2-nanosheet-modified upconversion nanoparticles." *Journal of the American Chemical Society* 133.50 (2011): 20168-20171

Aims and Subgoals

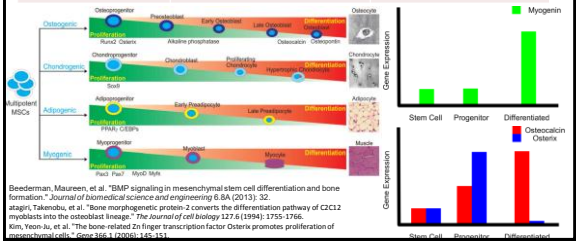
- Aim 1: creation of gelatin nanofiber-NS hybrid material
- Aim 2: *in vitro* myogenesis and osteogenesis
- Aim 3: *in vivo* transplantation of fibers with cells



Development of Platform

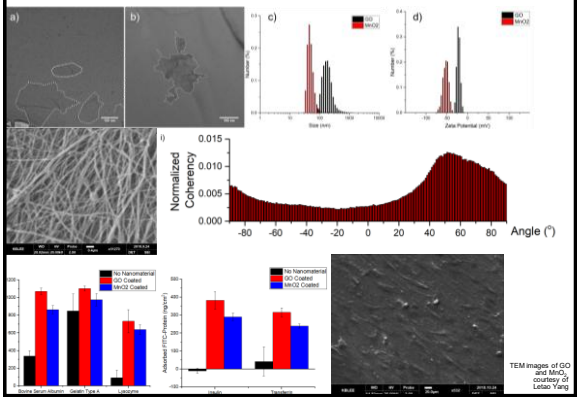
- System where nanomaterial and adsorbed molecule can be easily swapped

	Peptide or Small Molecule	Differentiation
Insulin and Transferrin	Peptide- Soluble Cue	Myogenesis
BMP-2	Peptide- Soluble Cue	Osteogenesis
Fibronectin	Peptide- Extracellular Matrix	Osteogenesis
Dexamethasone	Small Molecule	Osteogenesis



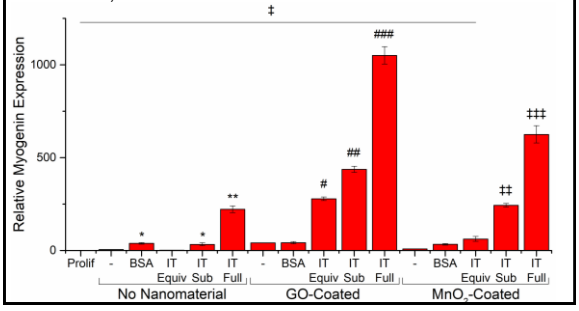
Beederman, Maureen, et al. "BMP signaling in mesenchymal stem cell differentiation and bone formation." *Journal of biomedical science and engineering* 6.8A (2013): 32.
atagiri, Takenobu, et al. "Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage." *The Journal of cell biology* 127.6 (1994): 1755-1766.
Kim, Yeon-Ju, et al. "The bone-related Zn finger transcription factor Osterix promotes proliferation of mesenchymal cells." Gene 366.1 (2006): 145-151.

Aim 1: Substrate Characterization



Aim 2: Myogenesis PCR

- ***Equivalent IT in media*** (No nanomaterial – 0.26 µg Transferrin, GO – 2.39 µg Insulin and 1.97 µg Transferrin , MnO_2 – 1.81 µg Insulin and 1.50 µg Transferrin)
- ***Full IT in media*** (literature recommended- 5 µg/ml insulin, 2.75 µg/ml transferrin)



Aim 2: Myogenesis PCR

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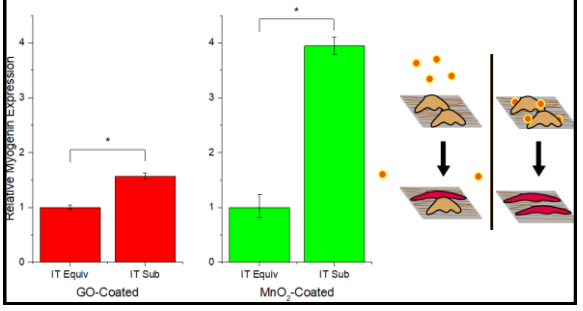
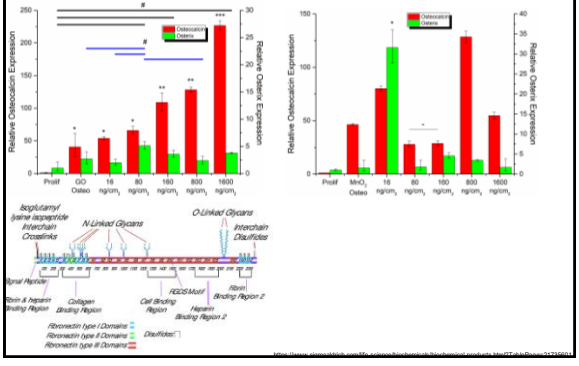


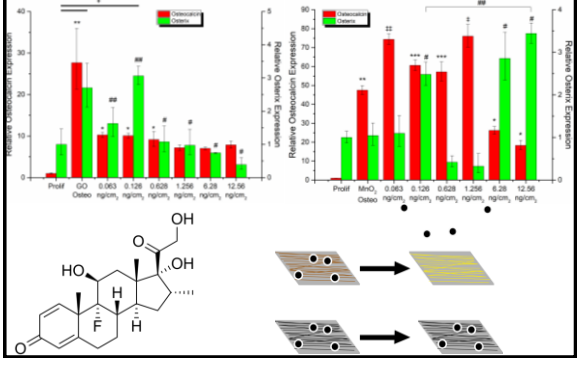
Aim 2: Dexamethasone and Fibronectin

- Fibronectin



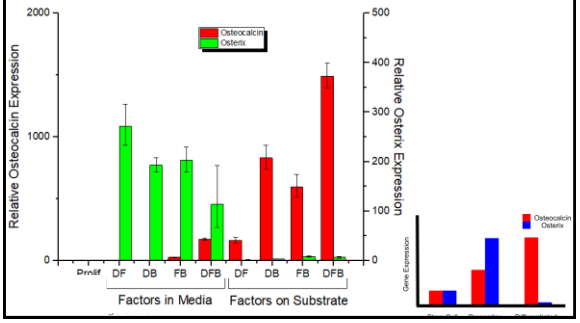
Aim 2: Dexamethasone and Fibronectin

- Dexamethasone



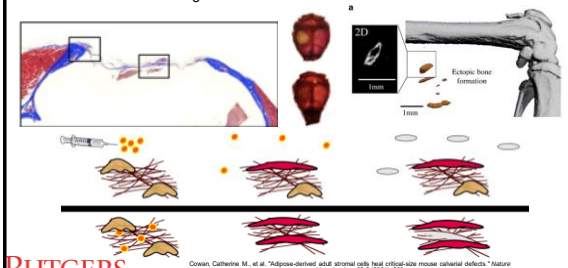
Aim 2: Combination Osteogenesis

- **Only MnO_2** because dexamethasone with GO ineffective
  - **Dexamethasone (1.256 ng/cm²), Fibronectin (800 ng/cm²), BMP2 (32 ng/cm²)**



Future Work

- Immunostaining for myosin and osteocalcin
- *In vivo* demonstration (critical size defect)
  - Reduction of off-target effects from BMP2



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Cowan, Catherine M., et al. "Adipose-derived adult stromal cells heal critical-size mouse calvarial defects." *Nature biotechnology* 22.5 (2004): 560.

Sheyn, D., et al. "Ultrasound-based, nonviral gene delivery induces bone formation in vivo." *Gene therapy* 15.4 (2008): 257.

Heo, Soon Chul, et al. "Periostin accelerates bone healing mediated by human mesenchymal stem cell-embedded hydroxyapatite/tricalcium phosphate scaffold." *PloS one* 10.3 (2015): e0116898.

Questions or Comments?

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Development of Bioactive Collagen-based Materials for Tissue Engineering Applications

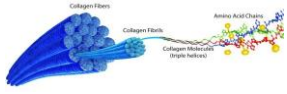
Yoliem S. Miranda Alarcón
PI: David I. Shreiber, PhD
Fall 2018

Outline

- Background and significance
- Project premise
- Experimental design & Aims
- Results
- Future directions
- Acknowledgements

Collagen

- **Collagen**, as a natural polymer, is a biomaterial of interest to the medical community due to its properties conferred by the protein's native structure, chemical composition (versatile), and abundance.
- Collagen's **triple helix domain** facilitates the interaction to a number of proteins and functions.



At 37ºC and pH of 7.4 collagen monomers self-assemble into a **fibrillar structure.**

Collagen provides mechanical support, necessary proteins and molecules to assist in the **wound healing** process.

Collagen functional modifications

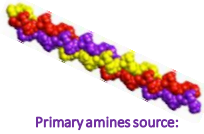
Collagen materials developed in Shreiber lab:

Grafted Molecule	Added functionality	Crosslinkers	Purification	Timeline
Bioactive peptides	Enhanced cell attachment	EDAC	Dialysis/Freeze drying	8 days
Methacrylic acid	Thermoreversibility and Photoactivity	EDAC/Sulfo-NHS		
Beta lactam antibiotics	Antibacterial	EDAC/Sulfo-NHS	Size Exclusion Chromatography/Protein concentrator/Freeze drying	4 days

Application

- Surgical site infections (SSI) affects in average 230,000 patients per year in the United States.
- Estimated annual cost for treatment of up to $10 billion.
- SSI is responsible for 38% of all infections.

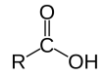
Project Premise



Primary amines source:
Collagen type-I

EDAC/Sulfo-NHS
Coupling agents

+



Carboxylic acid source:
β-Lactam antibiotics

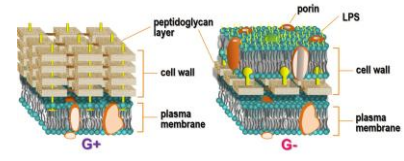
Reaction Optimization
pH & solubility
Temperature
Stoichiometry
Reaction chemistry

Our **proposed hypothesis** is that carboxyl-containing antibiotics grafted to collagen type-I would provide antibacterial protection to afford a scaffold for prevention of surgical site infections.

Considerations for antibiotics

- –COOH functionalized
- Mechanism of action **does not require** to enter the cell.
- Inhibition of cell wall synthesis
  - i. target peptidoglycan layers

β-Lactam antibiotics



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Aims

Aim 1: To synthesize and purify a collagen-based hydrogel wound dressing with antibacterial properties.

Aim 2: To characterize a collagen-based hydrogel wound dressing with antibacterial properties.

Aim 3: To test the efficacy of the antibacterial scaffolds *in vitro* and *in vivo.*

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Aims

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Aim 3: To test the efficacy of the antibacterial scaffolds *in vitro* and *in vivo.*

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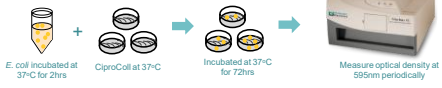
Experimental design: Aim 1

Objective: Establish protocols to graft and purify a variety of carboxyl-containing antibiotics to collagen type-I.

Grafted antibiotic	Starting collagen concentration	Purification method	Timeline
Ticarcillin	2.5 mg/mL	SEC/Concentrator	4 days
Ticarcillin	3.75 mg/mL	SEC/Concentrator	4 days
Carbenicillin	2.5 mg/mL	SEC/Concentrator	4 days
Carbenicillin	3.75 mg/mL	SEC/Concentrator	4 days

Bacterial Assessment:



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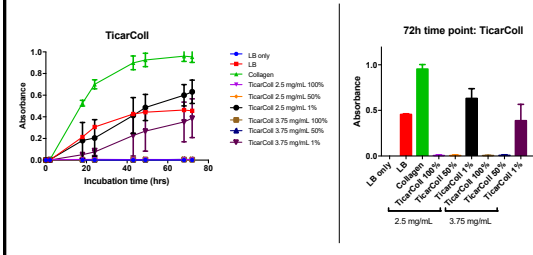
Results: Aim 1

Objective: Establish protocols to graft and purify Ticarcillin antibiotic to collagen type-I.



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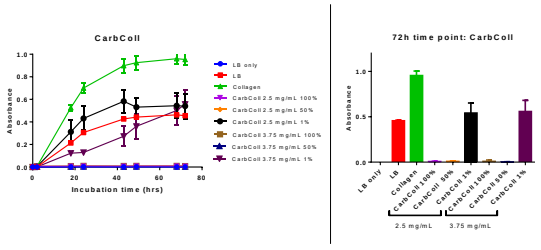
Results: Aim 1

Objective: Establish protocols to graft and purify Carbenicillin antibiotic to collagen type-I.



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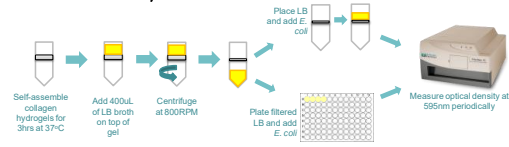
Experimental design: Aim 1.2

Objective: Confirm the removal of 'free antibiotic' from self-assembled hydrogel.

- Self-assembled hydrogels were flushed with LB using 0.22um microcentrifuge filters.
- Filtered LB was exposed to *E. coli* and OD measurements were taken at 0h, 24h, and 72hrs.
- BCA assay was carried out to confirm there was no filtered protein.

'Free antibiotic' assay:



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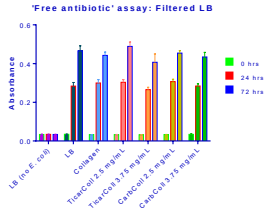
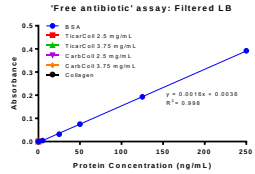
Results: Aim 1.2

Objective: Confirm the removal of 'free antibiotic' from self-assembled hydrogel.

Bacterial Analysis



Protein Analysis



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Aim 2

Aim 1: To synthesize and purify a collagen-based hydrogel wound dressing with antibacterial properties.

Aim 2: To characterize a collagen-based hydrogel wound dressing with antibacterial properties.

Aim 3: To test the efficacy of the antibacterial scaffolds *in vitro* and *in vivo*.

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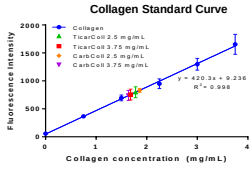
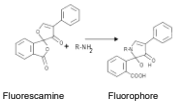
Experimental design: Aim 2.1

Objective: To confirm the ability of modified collagen to self-assemble into a hydrogel and evaluate the degree of conversion.

- Mechanical properties of hydrogels were assessed with rotational rheometry: Shear modulus was analyzed with change in temperature (4°C to 37°C).
- Fluorescamine fluorophore was employed to study the reaction efficiency.

Grafting Efficiency Assay:





Fluorescamine Fluorophore

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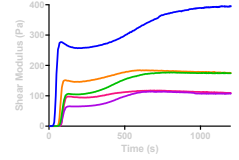
Results: Aim 2.1

Objective: To confirm the ability of modified collagen to self-assemble into a hydrogel and evaluate the degree of conversion.



Rheological Analysis

Grafting Efficiency Analysis

Collagen material	Collagen concentration (mg/mL)	Grafting efficiency %
TicarColl 2.5 mg/mL	1.78	52.6
TicarColl 3.75 mg/mL	1.68	55.2
CarbColl 2.5 mg/mL	1.86	50.4
CarbColl 3.75 mg/mL	1.64	56.2

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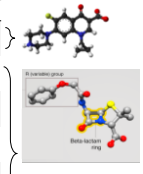
Proposed Antibiotics

Antibiotic	Solubility	Mechanism of action	Outcome
Ciprofloxacin	10 mg/mL 0.1N HCl	Inhibiting DNA gyrase, topoisomerase II and IV.	Did not work. Mechanism of action requires release of antibiotic.
Ampicillin	50 mg/mL 1N HCl	B-lactam family: Cell wall inhibition	Did not work, poor grafting efficiency.
Amoxicillin	50 mg/mL 1N HCl	B-lactam family: Cell wall inhibition	Did not work, too acidic (pH <1).
Penicillin G	100 mg/mL H_2O	B-lactam family: Cell wall inhibition	Did not work, the antibiotic was not potent enough.
Carbenicillin	50 mg/mL H_2O	B-lactam family: Cell wall inhibition	Grafted material inhibited bacteria
Ticarcillin	50 mg/mL H_2O	B-lactam family: Cell wall inhibition	Grafted material inhibited bacteria
Cephalosporin: B-lactam family sub-group			To be tested



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Future Directions

- HPLC will be carried out to confirm the removal of 'free antibiotic' and evaluate the degree of conversion of the modified collagen hydrogels.
- Evaluate the conformational structure of the engineered material through Circular Dichroism.
- Coupling of grafted antibiotic to PBP's transpeptidase domain will be confirmed.
- Efficacy of the antibacterial scaffolds *in vitro* and *in vivo* will be tested.

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Acknowledgements

-Dr. David I. Shreiber
-The Shreiber Group
-Nancy E. Hernández, PhD
-Gormley's Lab

NIH National Institute of General Medical Sciences
NIH T32 GM008339

NSF

Questions?

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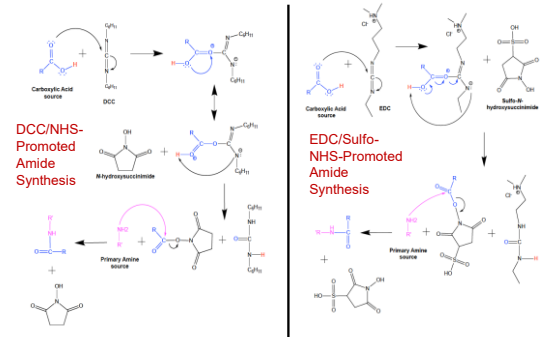

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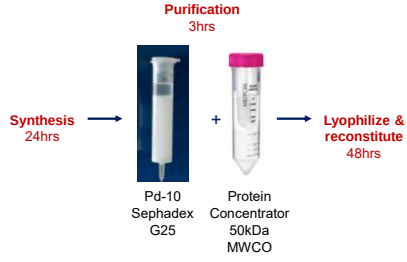
TicarColl Purification



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Experimental Design: Synthesis Conditions

Objective: Study synthesis conditions to optimize the covalent attachment of B-lactam antibiotics to collagen and remove any unbound material.

Grafted antibiotic	Starting collagen concentration	Condition
Ticarcillin	3.75 mg/mL	Control*
		PD-10 + Dialysis
		Post-lyo wash
		25% antibiotic
Carbenicillin	3.75 mg/mL	Control*
		PD-10 + Dialysis
		Post-lyo wash
		25% antibiotic

*Control: PD-10 column followed by protein concentrator and dialysis

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9. https://optn.transplant.hrsa.gov/?TB_iframe=true&width=914.4&height=921.6

WHY TEACH ETHICS?

Have you noticed it too?

US news

Trump officials oppose funding museum for victims of Tuskegee syphilis study

Justice department fighting use of unclaimed money from settlement for museum honoring black men who were not given treatment for disease

Associated Press in Birmingham, Alabama

Saturday 24 June 2017 [illegible] EDT

Tuskegee syphilis study participant Fred Simmons on his way to the White House in 1997, where Bill Clinton issued an apology to survivors of the study. Photograph: Dave Martin/AP

WELL THIS ISN'T WORTH A FUSS, IS IT?

TRUMP TRACKER

Science 1/12/18

Political review of grants

A senior adviser at the U.S. Department of the Interior will review certain grants and cooperative agreements of $50,000 or more to universities and nonprofit groups to ensure they "better align" with the priorities of President Donald Trump's administration. The move, which follows a similar decision last summer by the Environmental Protection Agency, was first reported by *The Washington Post*, citing a 28 December 2017 memo. The new approval process appears to be without precedent within the Interior Department, the *Post* reported. The priorities of Secretary of the Interior Ryan Zinke include securing the U.S. southern border and "utilizing our natural resources," according to an accompanying memo.

The Washington Post

Democracy Dies in Darkness

Government scientists blocked from the biggest meeting in their field

By Sarah Kaplan December 22, 2017

NEW ORLEANS — Hundreds of U.S. Geological Survey scientists were missing from the biggest conference in their field this month.

Typically, some 450 researchers from the nation's top natural resources and natural hazards agency attend the annual meeting of the American Geophysical Union, the largest gathering of Earth, space and climate scientists in the world.

But in the weeks before this year's conference, the Interior Department — which oversees the USGS — issued a new cap on attendance: No more than 199 employees across the department could travel to the meeting …

… one USGS scientist who was denied approval to attend the AGU conference just 10 days before the meeting said the crackdown on attendance amounted to the Interior Department "telling us we can't do our jobs … It's in my position description that I am to conduct research and disseminate that research…"

11:22 AM

SCIENTIFIC AMERICAN.

Observations

Trump to CDC: These 7 Words Are Now Forbidden

The administration's war on science takes a dangerously Orwellian turn

By Gleb Tsipursky on December 16, 2017

Do you want your medical treatm[illegible]mp administration disag[illegible] agency, th[illegible] C), from th[illegible] "Cattlement," "diversity," "[illegible]ciat[illegible] ie[illegible] [illegible]ased" and "science-based."

CENSORED

11:22 AM

NATURE | NEWS

Sea versus senators

North Carolina sea-level rise accelerates while state legislators put the brakes on research.

Leigh Phillips

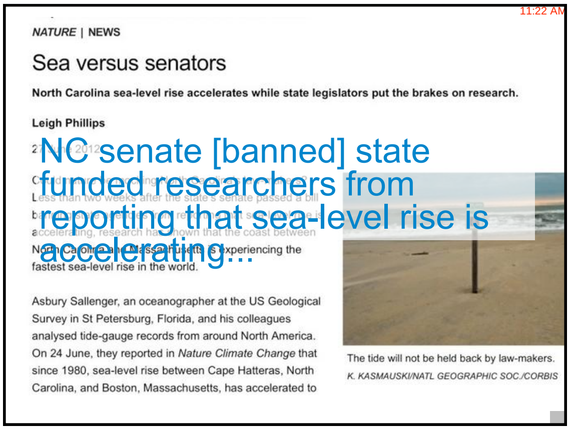
27 June 2012

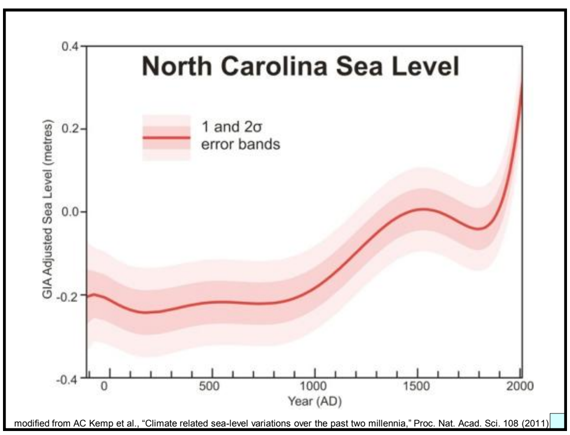
Could nature be mocking North Carolina's law-makers? Less than two weeks after the state's senate passed a bill banning state agencies from reporting that sea-level rise is accelerating, research has shown that the coast between North Carolina and Massachusetts is experiencing the fastest sea-level rise in the world.

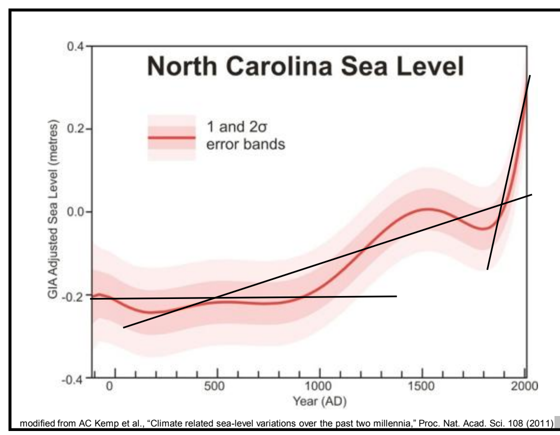
The tide will not be held back by law-makers.

K. KASMAUSKI/NATL GEOGRAPHIC SOC./CORBIS

Asbury Sallenger, an oceanographer at the US Geological Survey in St Petersburg, Florida, and his colleagues analysed tide-gauge records from around North America. On 24 June, they reported in *Nature Climate Change* that since 1980, sea-level rise between Cape Hatteras, North Carolina, and Boston, Massachusetts, has accelerated to



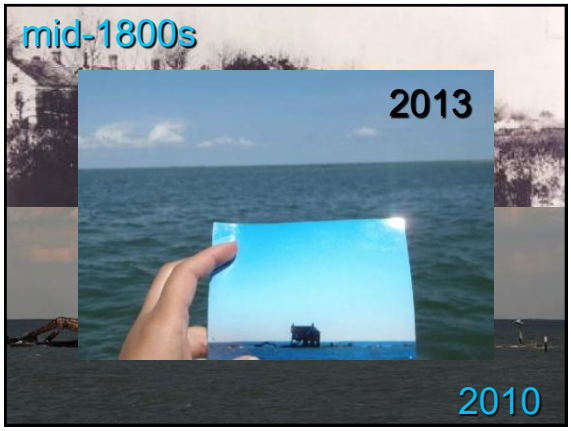




SO WHY STUDY ETHICS?

Because ethics matters…

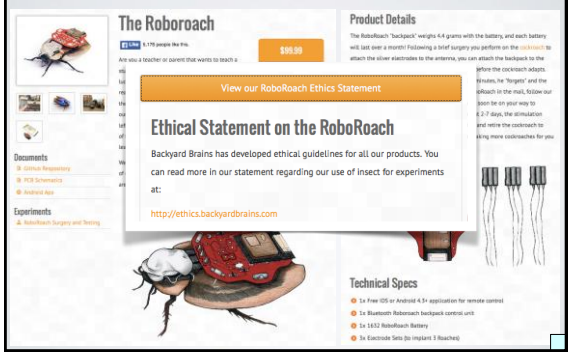
How does ethics matter?

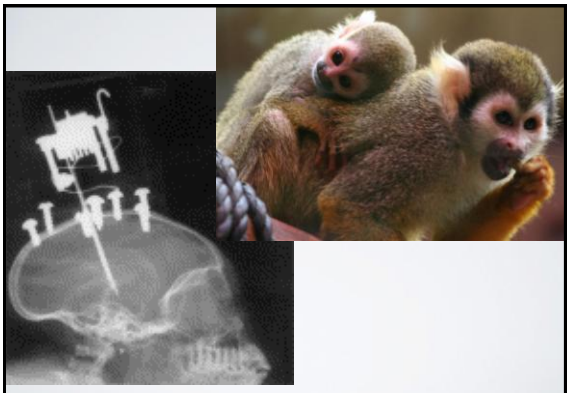


(staff writers) "Cockroach Stability," Science 297 (2002) 1643

CASE 3: TED LECTURE BY PAUL ROOT WOLPE:

HTTPS://WWW.YOUTUBE.COM/WATCH?V=OVV7V2XYJAI





Ludvig et al., "Single-cell recording from the brain of freely moving monkeys," J. Neurosci. Methods 106 (2001) 179-87

Later lecture:

TED talk by Paul Root Wolpe:

"It's time to question bioengineering"

www.youtube.com/watch?v=0vV7v2XYJAI

A different engineering ethical issue:

July, 2016

New, 2018:

BOOKS *et al.*

ARTIFICIAL INTELLIGENCE

The automated battlefield

A sober treatise on the future of warfare warns of the perils of autonomous robotic combatants

By George Lucas

U.S. Marines conduct a foot patrol with the Modular Advanced Armed Robotic System on 9 July 2016.

system malfunction and thus very likely averted a nuclear holocaust. Despite subsequent advances in artificial intelligence capabilities, none is sufficient to equip an automated weapons-delivery platform to make the kinds of discerning judgments that humans have made and continue to make routinely in such circumstances.

Machines lack the concerns, empathy, and self-consciousness that factor into normal human situational awareness. Thus, despite numerous advantages, Scharre marshals his engagingly detailed accounts of these weapons systems to argue that a policy of blindly arming and deploying autonomous robots in armed conflict would constitute an error fraught with peril.

Debates over the future of military robotics have raged for more than a decade among the delegates to the United Nations' periodic conventions on Certain Conventional Weapons (CCW) in Geneva, while members of ICRAC (International Committee for Robot Arms Control), including eminent Irish roboticist Noel Sharkey, have lobbied strenuously against the further development or deployment of such weapons.

ARMY OF NONE

Army of None: Autonomous Weapons and the Future of War
Paul Scharre
Norton. 2018. 400 pp.

REFERENCE

1. P.W. Singer, Wired for War: The Robotics Revolution and Conflict in the 21st Century (Penguin, London, 2009).

10.1126/science.aas9074

SCIENCE

HISTORY OF ETHICS OF ENGINEERS:

Wernher von Braun &
Nazi colleagues, 1941

WERNHER VON BRAUN:
WWII ACCOMPLISHMENTS

SS Sturmbannführer (Major)

Responsible for the design of the V-2 rocket

AFTER WWII:

Wernher von Braun &
U.S. Army General J.B. Medaris,
ca. 1955

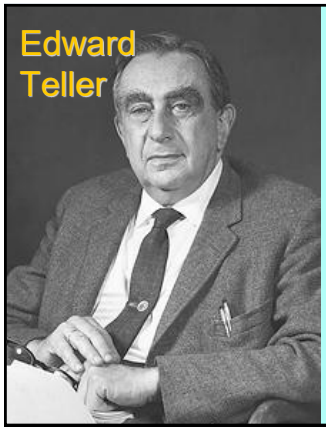
finally VP Fairchild Industries, maker aircraft & automotive parts.

WERNHER VON BRAUN:
POST-WWII
ACCOMPLISHMENTS

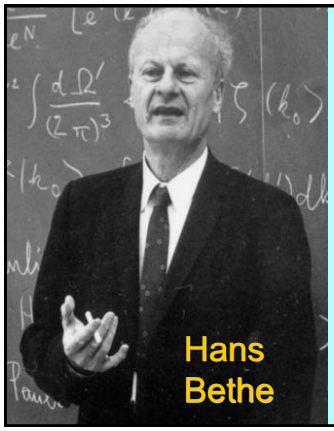
Technical director of the US Army's Ballistic Missile Agency

Responsible for the Pershing missile,

and the Saturn rocket (used in moon landing)



"The pursuit of knowledge and the expansion of human capabilities are intrinsically worthwhile."



"... to cease and desist from work creating, developing, improving and manufacturing further nuclear weapons – and, for that matter, other weapons of potential mass destruction such as chemical and

"Perfection of means and confusion of goals seem, in my opinion, to characterize our age. "

- A. Einstein, 1950

Q1) Are scientists and engineers obliged to consider the ethics and morality of their work?

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Q2) Do you believe that any technology can be used for good or evil, so no technology should be taboo?

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Q2) Do you believe that any technology can be used for good or evil, so no technology should be taboo?

Q3) Would you ever argue that “If I don’t do it, someone else will?”

Q1) Are scientists and engineers obliged to consider the ethics and morality of their work?

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Q4) Are there topics that you personally would refuse to work on for ethical or moral reasons?

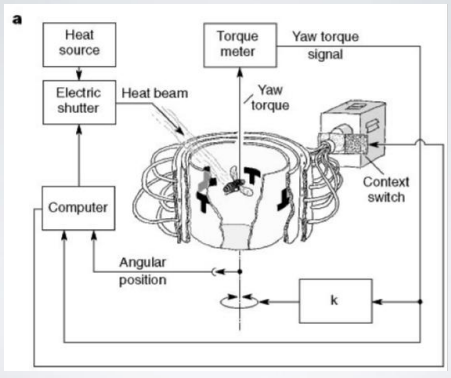
Q1) Are scientists and engineers obliged to consider the ethics and morality of their work?

Q2) Do you believe that any technology can be used for good or evil, so no technology should be taboo?

Q3) Would you ever argue that “If I don’t do it, someone else will?”

Q4) Are there topics that you personally would refuse to work on for ethical or moral reasons?

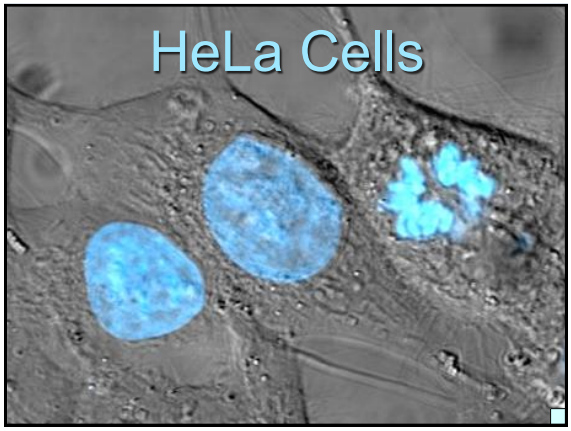
Q5) Would you sign Bethe’s Hippocratic oath, or do you side with Teller and feel that it is always preferable to gain more knowledge?



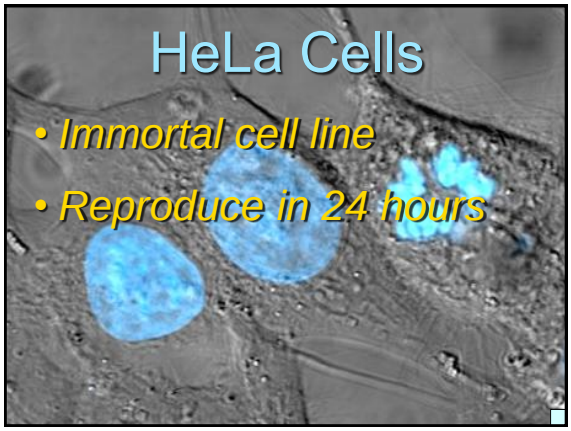
Liu et al., “Context generation in Drosophila visual learning requires the mushroom bodies,” Nature 400 (1999) 753-6

Who owns your body?

HeLa Cells

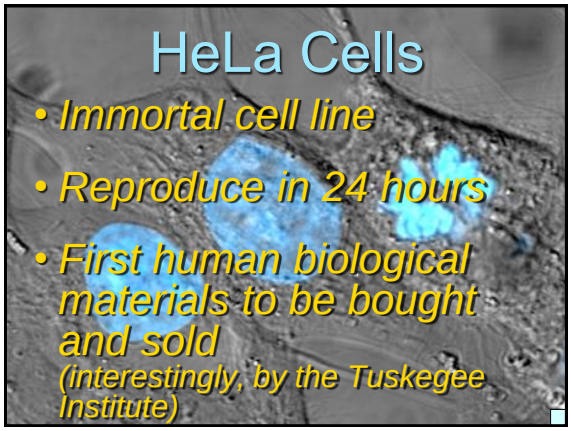


HeLa Cells



- *Immortal cell line*
- *Reproduce in 24 hours*

HeLa Cells



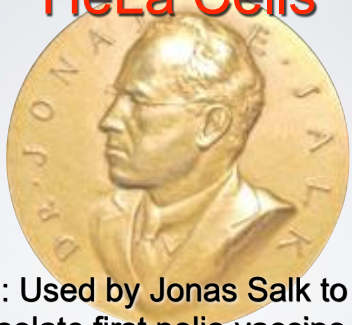
- *Immortal cell line*
- *Reproduce in 24 hours*
- *First human biological materials to be bought and sold*
  (interestingly, by the Tuskegee Institute)

HeLa Cells

1951: Removed from Henrietta Lacks by Howard Jones, MD

Given to George Gey, MD at JHU

HeLa Cells



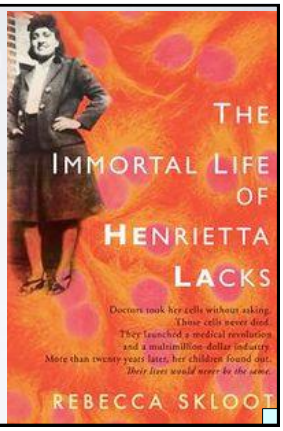
1952: Used by Jonas Salk to isolate first polio vaccine

HeLa Cells

2009: Over 60,000 research papers studying

- AIDS
- Cancer
- Cloning
- Gene mapping
- Vaccines
- Toxicity
- ...

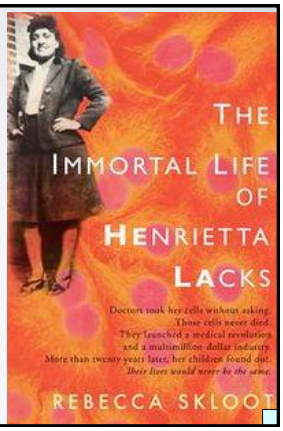
1974: Researchers worldwide discovered that HeLa cells had migrated to contaminate other cultures.



1974: Researchers worldwide discovered that HeLa cells had migrated to contaminate other cultures.

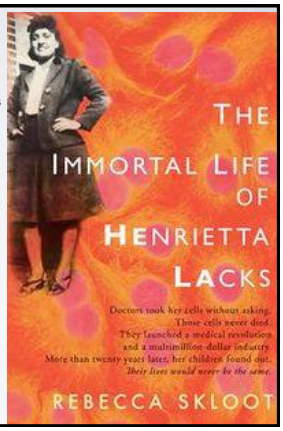
1975: family contacted for tests to distinguish HeLa from other cells.

Family told tests



HeLa:

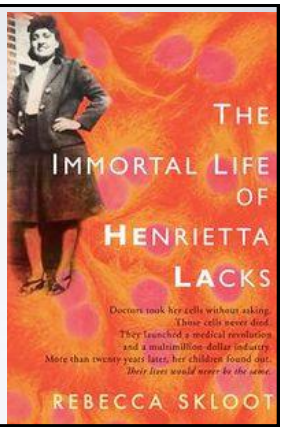
Q1) Informed consent: Ms. Lacks was informed of her treatment. What is wrong with using something that someone else has thrown away?



HeLa:

Q1) Informed consent: Ms. Lacks was informed of her treatment. What is wrong with using something that someone else has thrown away

Q2) Doesn't Ms. Lacks own her own cells?
- if yes, can't the state declare eminent domain?
- if no, why do we need donor cards?



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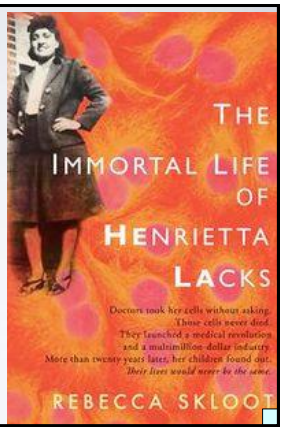
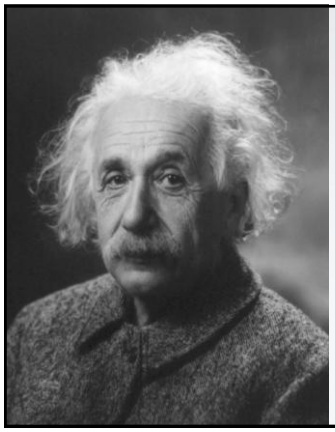
"... you can't buy and sell organs, that's illegal. But you can sell blood. You can sell human eggs and sperm. But you can't sell your kidney. And apparently, you can't sell your cells, you give those away."

- Ruth Faden, JHU





“Our age is characterized by perfecting the means while confusing the goals.”

— Albert Einstein

REMEMBER THE TUSKEGEE STUDY?

In 1932, Public Health Service began a study in Macon County, Alabama, called the "Tuskegee Study of Untreated Syphilis in the Negro Male".

The study involved 399 black men with syphilis. Researchers told the men they were being treated for "bad blood," a local term used to describe ailments including syphilis.

The men did not receive treatment through the study.

In fact, the doctors made the decision to prevent their treatment and to track the men until death.

In exchange for taking part in the study, the men received free medical exams, free meals, and burial insurance.

Originally projected to last 6 months, the study went on for 40 years.

WELL GUESS WHAT…

RUTGERS

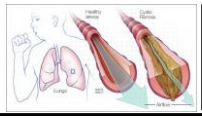
A Novel Genetic Engineering Approach: CRISPR-guided DNA Base Editing

Victor Tan
Shengkan Jin Lab
16th November 2018

RUTGERS

Introduction

- Genetic Engineering
  - "…manipulation of genome using biotechnology."
- Uses for genetic engineering
  - Crops
  - Genetic disorders



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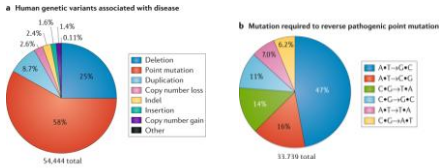
Genetic engineering and diseases



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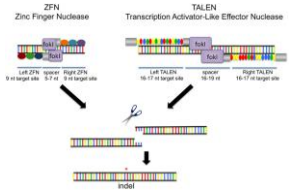
Previous gene editing technologies

- ZFNS, TALENS, CRISPR
  - Introduction of double stranded breaks (DSBs)

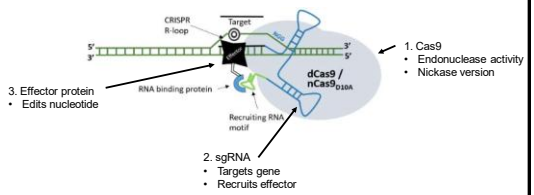


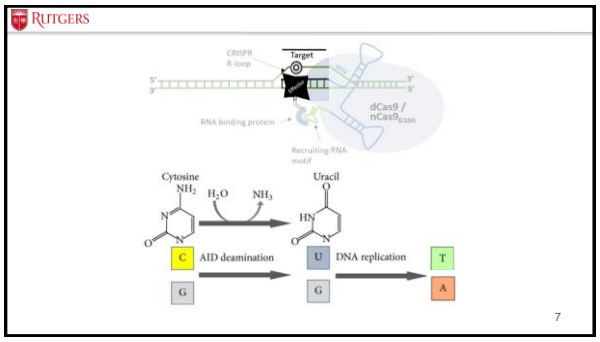
RUTGERS

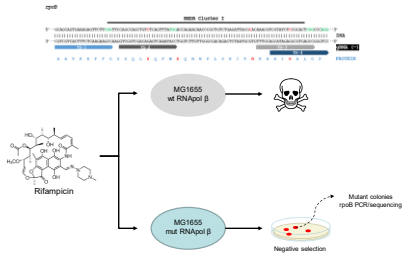
Mechanisms of gene editing: DNA repair

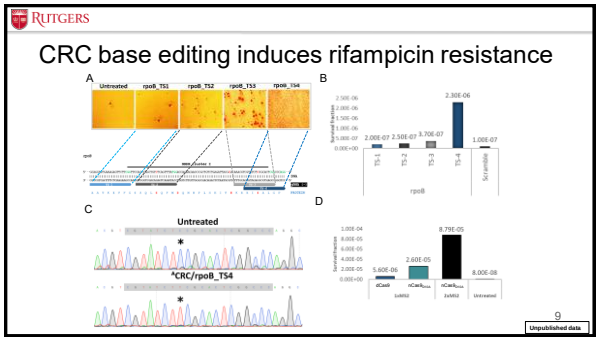
RUTGERS

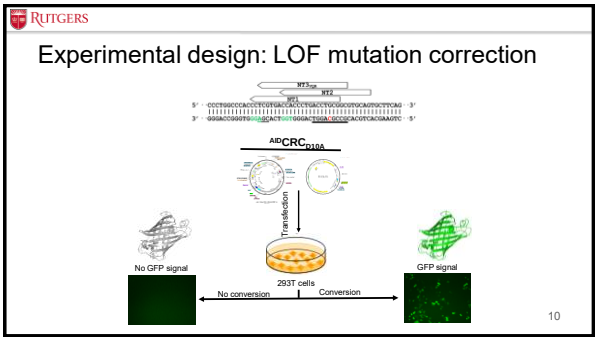
CRISPR/RNA Scaffold Mediated Correction/Modification system: CRC

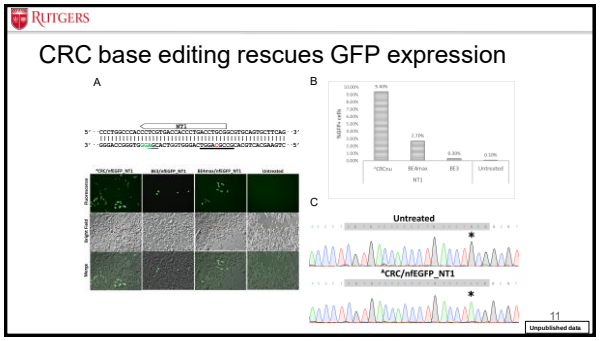


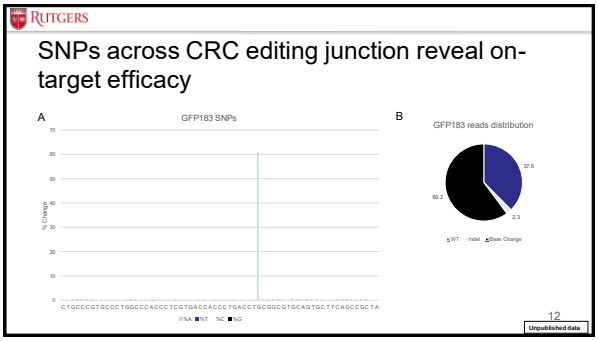

CRISPR R-loop
Target
dCas9 / nCas9D10A
RNA binding protein
Recruiting RNA motif
Cytosine
Uracil
AID deamination
DNA replication


Experimental design: Rifampicin resistance
MG1655 wt RNApol β
Rifampicin
MG1655 mut RNApol β
Mutant colonies rpoB PCR/sequencing
Negative selection


CRC base editing induces rifampicin resistance
Untreated
*CRC/rpoB_TS4


Experimental design: LOF mutation correction
No GFP signal
293T cells
GFP signal
No conversion
Conversion


CRC base editing rescues GFP expression
Untreated
*CRC/nEGFP_NT1


SNPs across CRC editing junction reveal on-target efficacy
GFP183 SNPs
GFP183 reads distribution

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Summary

- Limitations of current genetic engineering technologies
  - Off-target effects
  - Efficiency
  - Dependent on DNA repair mechanisms
- CRISPR/RNA Scaffold Mediated Correction/Modification system: CRC
  - Bypass double-stranded breaks
  - Base editing platform

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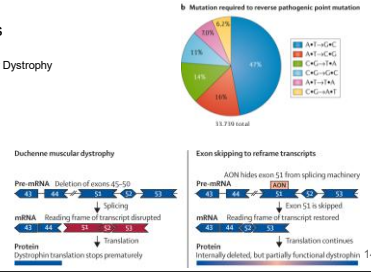
Future direction

- Disease models
  - CFTR
  - Duchenne Muscular Dystrophy
  - Huntington
  - Immunotherapy



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Acknowledgements

Jin Lab
- Dr. Shengkan Jin
- Dr. Juan Collantes
- Dr. Hanlin Tao
- Dr. Jingjing Guo

Funding
PhD Training Program in Biotechnology at Rutgers
NIH T32 GM008339

RUTGERS School of Engineering

Mesenchymal Stromal Cells for Treatment of Traumatic Brain Injury

Xiomara I. Perez
Biotechnology Training Program
November 16, 2018

Traumatic Brain Injury

TBI is a leading cause of morbidity and mortality worldwide

There are ~1.7 million new cases every year

~5 million Americans are dealing with subsequent cognitive deficits and motor dysfunctions

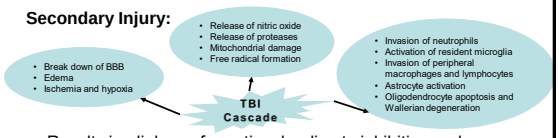
Annual expenditures of over $76 billion

Xiong et al (2017);

Physiological Response

Primary Injury:
- Mechanical injury which results in cell death and bleeding

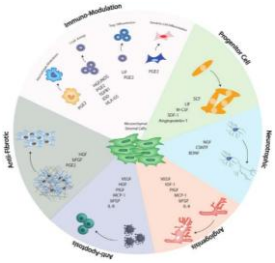
Secondary Injury:



- Results in glial scar formation, leading to inhibition and blockage of neuronal regeneration
- **Therapeutic interventions focus on preventing or slowing the progression of secondary injury in order to promote neuronal regeneration**

Currently, there is no FDA approved pharmacological treatment which is able to completely restore behavioral function

Mesenchymal Stromal Cells (MSC)



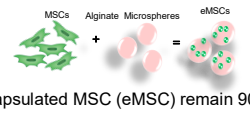
- MSC are adult stem cells commonly derived from bone marrow and adipose tissue
- Secrete anti-inflammatory cytokines and regenerative factors
- Used to treat acute organ injury
  - Liver
  - Kidney
  - Myocardial infarction

However, stem cell therapies require large quantities of cells due to low post-transplant viability and migration to other tissues.

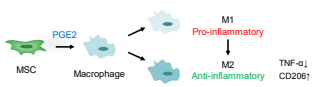
Barminko et al. (2011); Xiong et al (2017)

Previous Work: Cell Immobilization

- Our group has previously encapsulated MSC in alginate to maintain cell localization



- Alginate encapsulated MSC (eMSC) remain 90% viable for at least 2 months
- eMSC have an altered secretion profile, which includes upregulation of Prostaglandin (PGE2), a key anti-inflammatory mediator

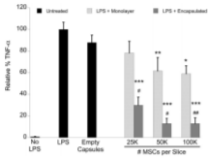


Barminko et al. (2011)

Previous Work: Neuro-inflammation Attenuation

Neuro-inflammation contributes significantly to tissue trauma and tissue degeneration

- Organotypic hippocampal slice cultures (OHSC) stimulated with lipopolysaccharide (LPS) followed by treatment with MSCs
- Measured TNF-α and PGE2 levels after 24 hrs



- In a dose-dependent manner, eMSCs significantly decreased TNF-α levels more effectively than monolayer MSCs
- Decrease in TNF-α is correlated with increased PGE2
- In a subsequent study, determined astrocytes were very responsive to eMSC treatment

MSC modulate inflammation in organotypic hippocampal slices and astrocytes stimulated with LPS

Stucky et al. (2015); Stucky et al. (2017)

*P < 0.02, **P < 0.002, ***P < 0.0001 compared with LPS + no treatment; #P < 0.01, ##P <0.002 compared with treatment with equivalent number of free MSC

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Neurotrophic Factors

MSC have been shown to secrete neurotrophic factors

- Neurotrophic factors support the survival of neuronal cells and can promote nerve fiber regeneration at injury sites

Neurotrophic Factor	Function
Nerve Growth Factor	• Promotes growth, differentiation, regeneration, and maintenance of neurons
Ciliary Neurotrophic Factor	• Promotes survival and development of neurons and glial cells
Glial Derived Neurotrophic Factor	• Promotes survival of neurons
Brain Derived Neurotrophic Factor	• Promotes the survival and differentiation of neurons • Induces axonal outgrowth and regeneration

However, basal MSC secretion of neurotrophic factors is not sufficient to promote functional recovery

Kumar et al (2012); Whone et al (2011); Razavi et al (2013); Razavi et al (2015); Hoffer et al (2016)

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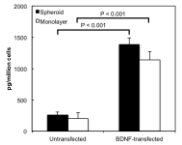
Transfected MSC BDNF Secretion

- Transfected human MSC with BDNF gene
  - Measured BDNF secretion 24hrs post transfection



Monolayer MSCs:
1.9 pg/ml BDNF per 10,000 cells

Transfected MSCs:
11.61 pg/ml BDNF per 10,000 cells

- *In vivo* mouse SCI model
  - Only transfected MSC spheroids had improved motor function recovery and reduced neuronal damage

There are still limitations with regards to cell viability, transfection efficiency, immunogenicity and technical skill requirement

Wang et al (2013); Uchida et al (2016)

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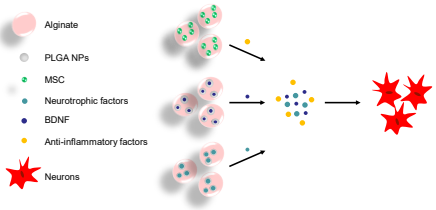
Hypothesis

We hypothesize that co-delivery of neurotrophic factors with eMSC will result in a combinational therapy with neuro-immunomodulatory properties and enhanced neuro-protective and neuro-regenerative efficacy



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Specific Aims

Aim 1: Optimize and characterize microencapsulated NPs for long-lasting, controlled delivery of BDNF.

Aim 2: Co-encapsulate BDNF-NPs with MSC in alginate microspheres and evaluate therapeutic efficacy *in vitro*.

Aim 3: Evaluate MSC co-encapsulated with BDNF-NPs as a TBI therapeutic *in vivo*.

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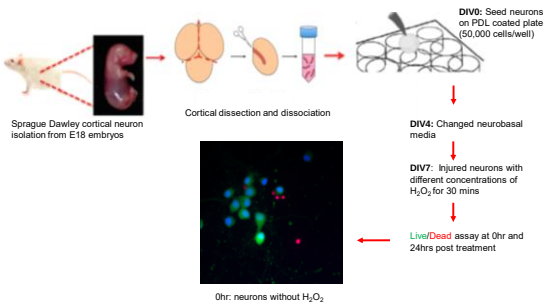
In Vitro Neuronal Culture



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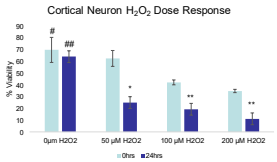
Preliminary Work: H_2O_2 Dose Response



* <.0001 compared to 0hrs at given concentration
** <.05 compared to 0hrs at given concentration
<.05 compared to all conditions at given time point, except 50μM
<.05 compared to all conditions at given time point

- Higher dosage of H_2O_2 decreases viability at 0hrs
- Lower dosage of H_2O_2 retains viability initially, but viability decreases after 24hrs

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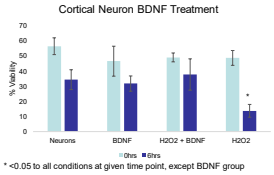
Preliminary Work: BDNF Treatment

- Neurons injured with 100μM H_2O_2
- Subsequent treatment with 25ng/ml BDNF for 6hrs
- Viability assessed via Live/Dead staining



* <0.05 to all conditions at given time point, except BDNF group

- At 0hrs, viability remained consistent between groups
- At 6hrs, groups without BDNF treatment had lower viability

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Future Work: Aim 1

- Dose response with glutamate to induce *in vitro* neuronal injury
- Dose response with BDNF treatment on injured neurons
  - Preliminary studies indicate that free MSC and eMSC secrete insufficient levels of BDNF for therapeutic benefits
- Diffusion and stability studies of BDNF loaded NPs encapsulated in alginate microspheres
  - Currently evaluating diffusion studies of encapsulated protein

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Future Work: Aim 2

- Treatment of injured neurons with MSC or eMSC +/- BDNF
  - Preliminary data suggests that treatment with MSC alone does not induce neuroprotection
- Evaluate functionality of MSC encapsulated with NPs
  - Preliminary data indicates that MSC remain viable for at least 48hrs post-encapsulation with empty NPs
- Co-encapsulate BDNF loaded NPs with MSC in alginate

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Acknowledgements

- Elisheva Strauss
- Mollie Davis
- Firestein Lab
- Dr. Rene Schloss
- Dr. Martin Yarmush
- Yarmush and Berthiaume Labs

Questions and Suggestions?

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The National Institute of General Medical Sciences (NIGMS)
NIH T32 GM008339

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Solving the structural activation mechanism of the Reelin signaling pathway

Liam Turk

Topics in Biotechnology

30 November 2018

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Presentation guide

1. Background
   1. Neocortex development requires Reelin
   2. Reelin disturbances and disease
   3. Biochemical and signaling profile of Reelin
2. Outstanding questions and data
   1. Reelin apoprotein structural data
   2. Complex formation between Reelin and receptors
   3. Biochemical strategy to explore signal activation question
3. Summary and future work

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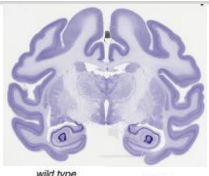
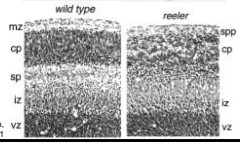
Neocortex development requires reelin

- Neocortex: highly organized, outer subsection of cortex
- Involved in higher order cognitive functions – crucial for evolution
- Discrete layers within the neocortex
- Proper lamination dependent on Cajal-Retzius cell secretion of Reelin.



Allen Brain Atlas



Rice and Curran, 2001

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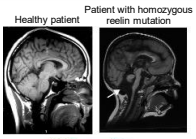
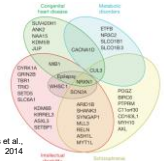
Reelin disturbances are associated with several diseases

- Patients with a rare homozygous reelin mutation display with lissencephaly and cerebellar hypoplasia.
- Altered levels of Reelin mRNA or protein are associated with neurological diseases, including autism spectrum disorders, schizophrenia, and depression.



Hong et al., 2000.



De Rubeis et al., 2014

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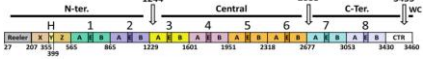
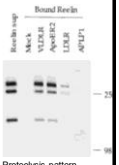
Reelin is a dimeric, secreted glycoprotein

- Eight repeats with proteolytic cleavage sites at 1244 and 2688
- Receptor binding site on repeat 6 – K2467.
- Disulfide linked dimerization site on repeat 5 – C2101.
- C2101A mutant monomer can bind but not initiate signaling.
- Signaling requires central fragment, repeats 3-6.





Proteolysis pattern seen with antibodies targeting N-terminus

D'Arcangelo et al., 1999

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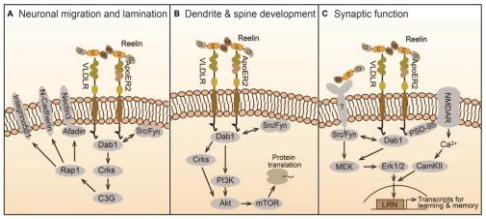
Reelin has distinct effects during different stages of development



A) Prenatal, B) early postnatal, and C) late postnatal functions (Lee & D'Arcangelo, 2016)

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Questions that interest us

- Can we solve the structure of a signaling competent Reelin construct?
  - So far only individual or pairs of repeats have been solved and nothing showing the dimeric form of Reelin.
- Can we use structure to infer the mechanism of activation ?
  - Dimeric Reelin binds its receptors and initiates a signal cascade, monomeric Reelin binds but does not signal. Stoichiometry or conformation/affinity?
- Can we provide biochemical evidence for the latter question?
  - Create a Reelin dimer comprised of two different chains and utilize a DAB1 phosphorylation assay.

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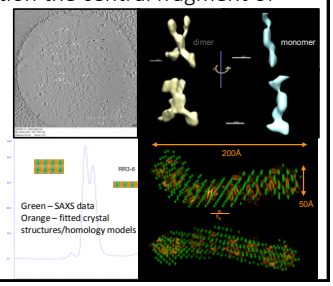
Structural characterization the central fragment of apo-Reelin.

- FLAG-Reelin-Fc construct purifies as monomer and dimer – DTT partially reduces disulfide.
- No gross conformational changes observed, protomers approximately 200Å x 50Å.
- Can observe overlapping 5th repeat – C2101.
- BioSAXS confirms size and shape of monomer.



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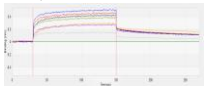
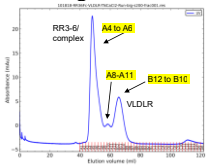
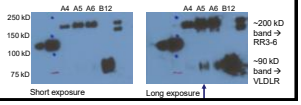
Complex formation of the central fragment of Reelin with its receptors

- Binding experiments show interaction occurring between Reelin and its receptor, ApoER2.





- Purified and mixed **equimolar amounts of central Reelin fragment and receptor, VLDLR**
- Blot of SEC fractions shows low levels complex formation.



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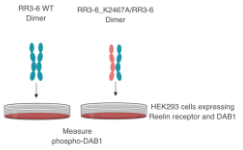
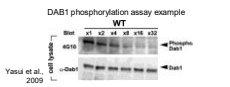
Does the Reelin dimer bind two receptor protomers to initiate signaling?

- Even if structure shows reelin binding two receptor protomers, this demonstrates capacity to bind, not necessity for signal transduction.
- Create two differentially tagged Reelin constructs
  - FLAG-Reelin (AA) (WT)
  - HA-Reelin_K2467A (abolishes receptor binding) (BB)
- Heterodimer FLAG-Reelin/HA-Reelin_K2467A (AB)





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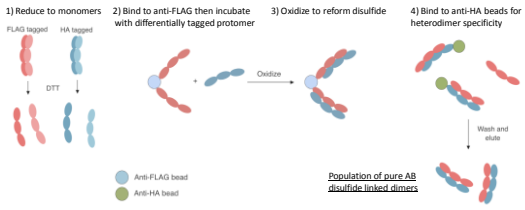
Schema to isolate heterodimers



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Summary

- Reelin secretion is imperative in early brain development and a healthy adult brain.
- The biologically active form of Reelin is a dimer that is linked on repeat 5, adopting an X-like conformation with each monomer having dimensions of approximately 200x50Å. No architectural intramolecular shifts upon dimer formation.
- SAXS supports preliminary cryo-ET data
- At current concentrations, we can observe low level complex formation between Reelin and VLDLR.

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Further work to be done

- Processing data to form higher resolution cryo-ET data (Xuyuan Kuang).
- Increase concentrations of purified Reelin constructs to favor complex formation --> cryo-ET, AUC, SAXS to understand stoichiometry and structure of the complex.
- ITC to probe differences in binding affinities of monomer vs dimer.
- Create Reelin heterodimer to see if signaling is reliant on the localization of two receptors or conformation of the dimer.
  - Reduction/Oxidation method or co-transfection/double cassette?

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Acknowledgements

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Wei Dai
Xuyuan Kuang

D'Arcangelo Lab
Gabriella D'Arcangelo
Valentina Dal Pozzo

Child Health Institute of New Jersey

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NIH T32 GM008339

NSF Award IOS-1755189

Cornell High Energy Synchrotron Source

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Biochemical strategy to isolate the activating

- Does the Reelin dimer bind two receptor protomers (2 VLDLRs, 2 ApoER2s) to initiate signaling?
- Create two differentially tagged Reelin constructs
  - FLAG-Reelin (AA)
  - HA-Reelin_K2467A (abolishes receptor binding) (BB)
- Monomerize both in reducing conditions --> break intermolecular disulfide at C2101 (A,B)
- Attach FLAG-Reelin to anti-FLAG beads and wash (beads-A)
- Incubate with HA-Reelin and change the redox environment to favor the recreation of intermolecular disulfide (beads-AB, beads-A)
- Elute and then incubate with anti-HA (AB-beads)
- Last elution should solely be our heterodimer FLAG-Reelin/HA-Reelin_K2467A (AB)

Electroresponsive hydrogel optimization for stem cell differentiation

Brandon Newton

11/30/2018

Background

- Traumatic injury is the leading cause of muscular morbidity in the United States for residents under age 44.
- Current Gold Standard:
  - 1. Harvest muscle tissue from another region of body
  - 2. Implant at site of injury (autograft)
- Effective for small muscular injuries but inefficient for large muscle defects.
  - Additional problems include donor site morbidity, high insurance costs.

Cell line of interest

- Mesenchymal stem cells
- Multipotent stem cells derived from mesoderm that are able to differentiate into hematopoietic stem cells, osteoblasts, chondrocytes, adipocytes, and myocytes.
- The Cell line to be used in this study is the Rabbit Bone Marrow Derived Mesenchymal Stem Cell.

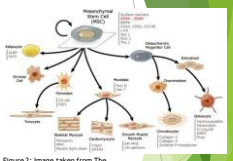


Figure 2: Image taken from The regeneration center of Thailand

Electro-responsive Hydrogels

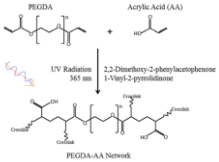
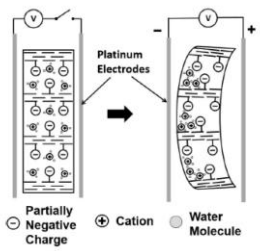


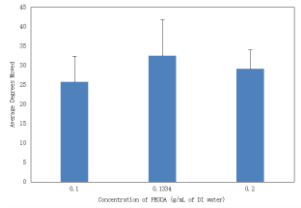
Figure 3: Crosslinking of PEGDA and Acrylic acid when exposed to photoinitiator solution.

IONS in Solution



MAETAC STUDY

- PEGDA was modified with the positively charged molecule 2-(methacryloyloxy) ethyl-trimethylammonium chloride (MAETAC) to provide its own positive ions.
- Since the movement of water is linked to the presence of positive cations in the solution a polymer that is positively charged may be more versatile.
- Modification should allow it to actuate in either an ionic solution or in pure water, allowing it to be active in a variety of solutions, including biological solutions.



Innovation utilizing ECM

- PEG based hydrogels have a lower cell attachment rate due to the formation of a hydrated surface layer that inhibits the adsorption of adhesion specific proteins such as fibronectin
- Use of Homogenized Muscular Extracellular Matrix (ECM)
  - Extracellular Matrices play a very important supportive role in the differentiation of stem cells and their precursor.
  - Help to control the concentration of both adhesive and signaling molecules in the local environment as well as contains a variety of cytokines which prevent cellular degradation.

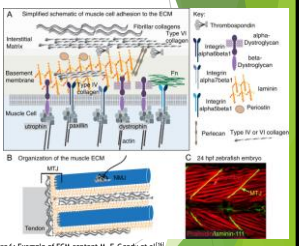
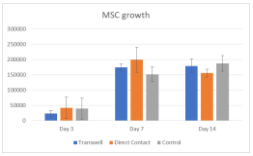


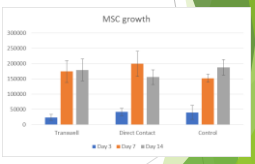
Figure 6: Example of ECM content M. F. Goody et al[16]

Innovation

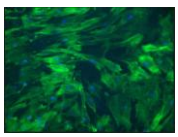
- One of the major issues with the previously mentioned electroresponsive hydrogels is that their influencing potential on differentiation is slightly ambiguous.
- Mesenchymal stem
  - remain viable for extended periods of time while on the hydrogels
  - difficult to define the amount of differentiation directly linked to the electroresponsive hydrogels.
- We aim to optimize the differentiation process regarding the hydrogels by modifying its components to maximize growth potential down specific lineages.

Presto Blue Assay

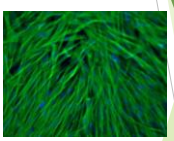




DAPI and Phalloidin staining day 14



MSC control at day 14



The transwell of cells exposed to muscle tissue ECM shows what appears to be spindle formation albeit not unidirectional

Innovation Utilizing Growth Factors

- Controlled release of growth factors
- TGF beta > able to bind with their respective receptors on the surface of stem cells > stimulates 3D skeletal muscle tissue engineering

Next Step

- Analyze Data for repeated study extended to 21 days.
- PCR

  Myosin Heavy, Myosin Light Chain, MyoD, Myogenin

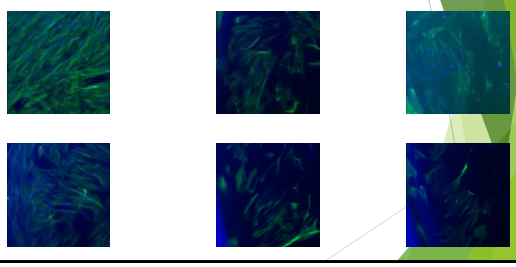
Thank You

Row A

Trans Well | Direct Contact | Control

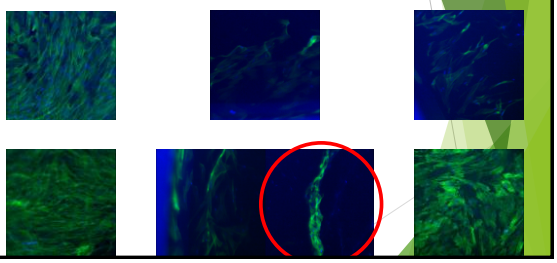
Row B

Trans Well | Direct Contact | Control



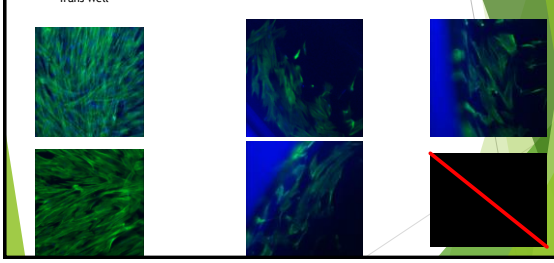
Row C

Trans Well | Direct Contact | Control



Row D

Trans Well | Direct Contact | Control



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16:125:604 Topics in Advanced Biotechnology
Spring 2019
Fridays, 9:00-11:00, BME Room 122

TOPIC	DATE	FACULTY MEMBER	STUDENT COORDINATOR	PRESENTERS	DISCUSSANTS
The Art and Science of the Biotech Program	Jan 25	Martin Yarmush	----	----	----
Bone Tissue Engineering	Feb 8	Joseph Freeman	Ileana Marrero-Berrios	Isabelle Perez Rahul Upadhya	Paulina Krzyszczyk Madison Godesky
"Off the Shelf" Cell Therapy	Feb 22	Biju Parekkadan	Anton Omelchenko	Emily DiMartini Alexandra Burr	Erika Davidoff Jeremy Anderson
TBD	March 8	Maribel Vazquez	Yoliem Miranda Alarcon	Caroline Wood Brandon Newton	Alison Acevedo Lauren Timmons
Neural Stem Cells	April 5	Zhiping Pang	Chris Rathnam	Andrew Boreland Skylar Chuang	William Pfaff Josh Leipheimer
Activating the Nrf2 Cell Defense Pathway	April 19	Ann Stock	Evelyn Okeke	Nisha Singh Jeffrey Luo	Ryan Guasp Mollie Davis
Mass Spectrometry Based Proteomics	May 3	Peter Lobel	Eve Reilly	Victor Tan Zach Fritz	Larry Cheng Matt Tamasi

- Faculty members prepare **a 30-minute presentation** to introduce both the overall topic and the papers to be discussed.
- Student coordinators should contact the assigned faculty advisor in order to identify two papers, **at least 3 weeks** prior to the session.
- Papers are sent to the presenters and Mary Ellen, and a first meeting is called to thoroughly review the papers (with the coordinator and the presenters).
- The group then meets with the faculty member to review draft slides and to answer any remaining questions.
- Each student prepares a 15 minute ppt presentation of their respective paper, and then meets with the coordinator to review the presentation. Attention should be given to both content and delivery.
- A final "dress rehearsal" is conducted with the entire group present (with the faculty member).
- The discussants read the papers, attend the dress rehearsal, and outline discussion points on each paper for the session according to prescribed guidelines.

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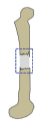
Injectable Polypeptide Hydrogel/Inorganic Nanoparticle Composites for Bone Tissue Engineering

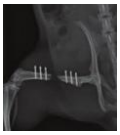
Rahul Upadhya
Rutgers University, Department of Biomedical Engineering

Biotechnology Training Program
Spring 2019 – Topics II
02/08/2019

Clinical Problem

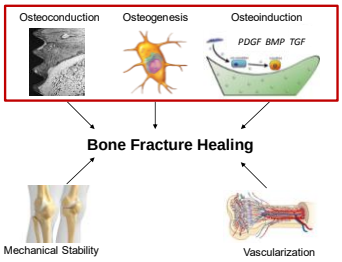
- **Critical bone defect:** defect greater than 1-2 cm and loss of at least 50% bone circumference
- Commonly leads to malformation, tumor formation, infection, and non-union without treatment
- Over 1 million reconstructive surgeries per year





Schemitsch, EH. *Journal of Orthopaedic Trauma* 2017.
Kadiyala, S et al. *Tissue Engineering* 1997.
Arinzeh, TL et al. *The Journal of Bone & Joint Surgery* 2003.

Mechanisms of Bone Healing



Common Bone Grafting Strategies

	Osteoconductive Bone growth on implant	Osteogenic Stimulation of progenitor cell	Osteoinductive Recruitment of preosteoblasts
Alloplast	+	-	+/-
Xenograft	+	-	-
Allograft	+	-	+/-
Autograft	+	+	+

Potential Concerns:

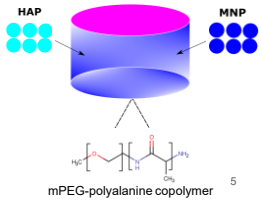
- Multiple surgical sites
- Lack of blood supply
- Limited availability

Research Objective

Goal: Synthesize bone scaffolds with useful mechanical properties and biocompatibility

Hypothesis: A hydrogel encapsulating hydroxyapatite (HAP) and Fe_3O_4 nanoparticles (MNP) will provide mechanical and biocompatible benefits

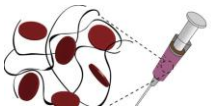
- Hydrogels mimic ECM
- HAP causes bone mineralization
- MNP has magnetic properties



mPEG-polyalanine copolymer

Experimental Methods

1. Hydrogel component characterization
2. Mechanical characterization
3. Cell viability study and gene expression



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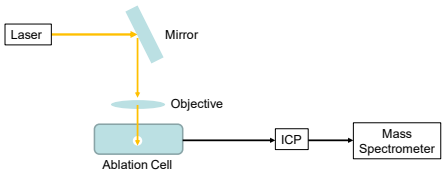
❶ Hydrogel Component Characterization

LA-ICP-MS Elemental Mapping



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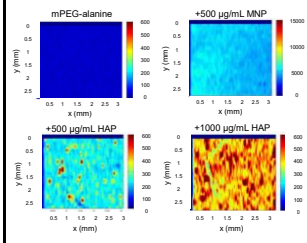
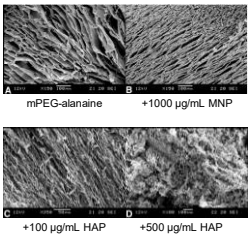
Hydrogel Material Characterization Results

LA-ICP-MS Elemental Mapping



Scanning Electron Microscopy (SEM)



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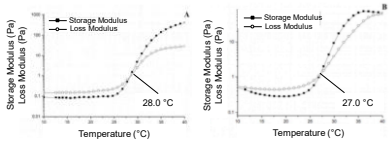
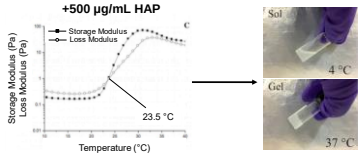
❷ Rheological and Mechanical Properties

mPEG-alanine **+1000 µg/mL MNPs**



+500 µg/mL HAP



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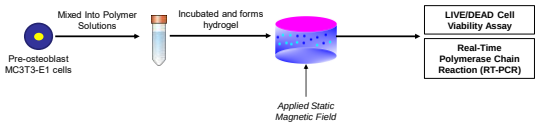
❸ Viability Assay and Gene Expression



Objective: Determine cell viability of pre-osteoblast cells and gene expression of osteopontin and alkaline phosphatase due to presence of magnetic field

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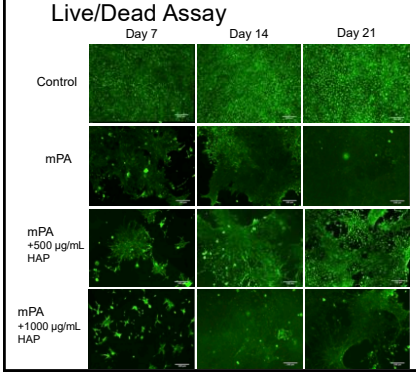
Live/Dead Assay



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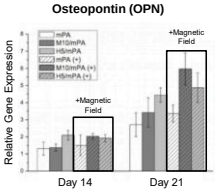
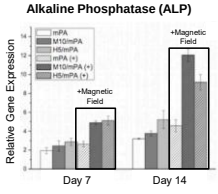
Gene Expression by RT-PCR

Osteopontin (OPN)



Marker for bone mineralization and remodeling

Alkaline Phosphatase (ALP)



Marker for osteoblast differentiation

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Conclusions

1. Higher concentration of encapsulated HAP shifted the hydrogel structure from plate-like to porous due to aggregation
2. Rheological features confirmed the formation of hydrogels at specific temperature ranges
3. Cell viability "optimal" at 500 µg/mL but quantification is needed
4. Gene expression studies demonstrate the benefit of static magnetic field on differentiation of pre-osteoblasts to osteoblasts

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Acknowledgements

Thank you:
-Xiomara Perez
-Ileana Marrero-Berrios
-Paulina Krzyszczyk
-Madison Godesky
-Dr. Freeman
-Dr. Yarmush and Dr. Stock

Rutgers Biotechnology Training Program

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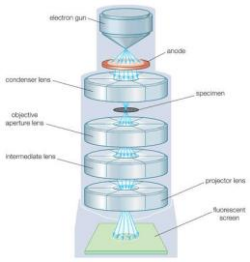
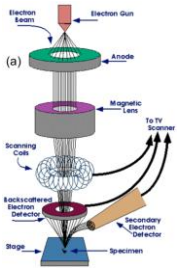
1 Imaging Hydrogel Components

Transmission Electron Microscopy (TEM)



Scanning Electron Microscopy (SEM)



RUTGERS School of Engineering

Scaffolds for Bone Tissue Engineering: State of the Art and New Perspectives

Livia Roseti, Valentina Parisi, Mauro Petretta, Carola Cavallo, Giovanna Desando, Isabella Bartolotti, Brunella Grigolo

Xiomara I. Perez
Biotechnology Training Program
February 8, 2019

Bone Fracture and Disease

Annually, more than **20 million** patients are affected by bone loss caused by disease or trauma

In the US, there are over **500,000** patients with bone defects

This leads to an annual expenditure of over **$2.5 billion**

If bone lesions do not heal properly, there is an increased with developing diseases such as osteoarthritis

Iaquinta et al (2019)

Bone Disease Treatments

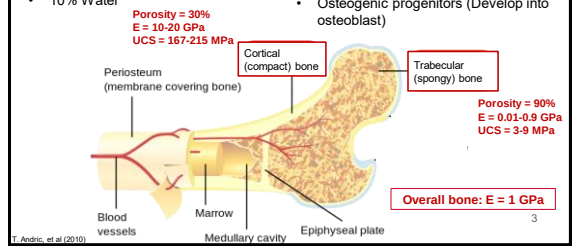
Treatment	Description	Disadvantage
Autograft (Gold Standard)	Bone tissue harvested from patient	• Surgical donor site • Increased risk of blood loss, infection, fracture, and cosmetic deformation at explanation site • Limited donor sites
Allograft	Bone tissue harvested from living donor or cadaver	• Risk of disease transmission • Risk of adverse immune response • Sterilization process compromises mechanical properties

Researchers have turned to tissue engineered bone grafts to overcome these limitations

Bone Structure and Composition

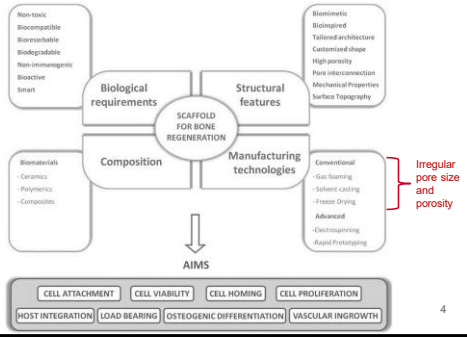
Extracellular Composition
- 70% Inorganic Mineral (Calcium Phosphate)
- 20% Organic Matrix (Collagen I)
- 10% Water

Cellular Composition
- Osteoblasts (Bone formation)
- Osteoclasts (Bone resorption)
- Osteocytes (Maintain mineral content)
- Osteogenic progenitors (Develop into osteoblast)



T. Andric, et al (2010)

Scaffold Strategies for Bone Regeneration



Rapid Prototyping

Additive, layer-by-layer, fabrication process

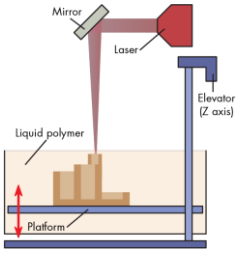
Computer Aided Design (CAD) → Program produces a data set readable by a fabrication machine (STL) → Manufacturing technology transforms the data set into the product

Bone Engineering: High precision, reproducibility, and controllable pore structure

Rapid Prototyping Techniques:
- Stereolithography (SL)
- Selective Laser Sintering (SLS)
- Bioprinting

Stereolithography

Curing polymer liquid layer by layer via an ultraviolet (UV) light



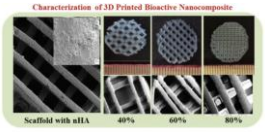
Advantage:
- High feature resolution
- High porosity
- Inexpensive equipment

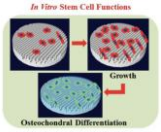
Disadvantage:
- Limited biomaterials
- Skin irritation and cytotoxicity
- Poor mechanical strength

Kerns et al (2015); Derakhshanar et al (2017)

Nanocrystaline Hydroxyapatite Based Scaffold

- **Polymer Solution:** hydroxyapatite (osteogenic), TGF-β1 (chondrogenic) and poly(ethylene glycol) diacrylate
  - Seeded human bone marrow derived mesenchymal stromal cells (hMSC) onto scaffolds



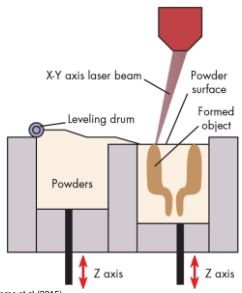


- hMSC adhesion, proliferation, and differentiation were improved *in vitro*
- **Compression Testing:** E ~ 13MPa, UCS ~24 MPa

Castro et al (2015)

Selective Laser Sintering (SLS)

High power laser binds powder particles layer by layer



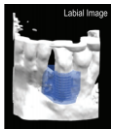
Advantage:
- High porosity
- Anatomically shaped scaffolds
- Availability of bio-ceramics and titanium

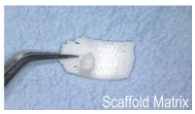
Disadvantage:
- Limited bio-polymers due to high operating temperature

Kerns et al (2015)

Bioresorbable Polycaprolactone (PCL) Scaffold

- CT scan was utilized to design model
- Prior to implantation, scaffold was immersed in a solution of recombinant human platelet-derived growth factor

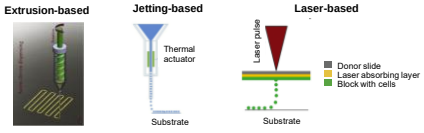




- After 12 months there was no indication of chronic inflammation or rejection, but scaffold failed at 14 months
  - Biomaterial not suitable for this application

Rasperini et al (2015)

Bioprinting

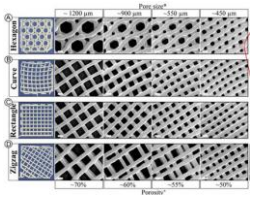


	Extrusion	Jetting	Laser
Biomaterials	High	Limited	High
Nozzle-clogging	Yes	Yes	No
Speed	Slow	Fast	Medium
Cost	Moderate	Low	High
Cell viability	~90%	80/90%	<85%
Cell density	High	Low	Medium
Resolution	Low (100μm)	Medium (50μm)	High (10μm)

Derakhshanar et al (2017)

Ceramic Based Scaffold (Ink Based)

- Strontium–hardystonite–gahnite powder was synthesized and dispersed in water-based organic solution to make bio-ink



- **Compression Testing**: UCS ~110MPa (Hexagonal)

Discussion and Conclusion

- There are several considerations that need to be made for engineering bone-scaffolds
  - Biological requirements
  - Biomaterials
  - Mechanical properties
- Scaffold requirements change based on the application
  - Load bearing vs non-load bearing
  - Scaffold architecture
  - Manufacturing technique

Acknowledgement

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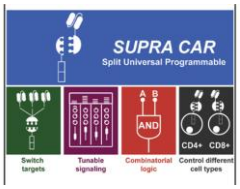
Ileana Marrero – Berríos
Paulina Krzyszczyk
Madison Godesky
Dr. Joseph Freeman

Biotechnology Training Program (NIH T32 GM008339)

Thank you!

Universal Chimeric Antigen Receptors for Multiplexed and Logical Control of T Cell Responses



Jang Hwan Cho, James J. Collins, Wilson W. Wong

Cell, 2018

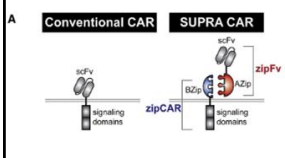
Presented by: Alexandra Burr

Conventional CAR

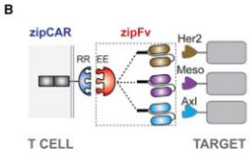
Unmet need: Current CAR systems

- Safety & efficacy
- Finely tune T cell activation
  - scFv and CD3 domain = simultaneous activation at a predetermined level
  - Resulting effect: CAR T cell-related toxicity
- Enhance tumor specificity
  - Fixed, high-affinity scFv = difficulty in discriminating against antigen density
  - Resulting effect: dangerous reactivity against healthy organs
- Independently control different signaling pathways
  - CD3 domain is fixed to one subset
  - Resulting effect: non-optimal T cell response
- On/off switch
  - Has been developed, but not combined with other features
  - Resulting effect: cytokine release syndrome

Design and Characterization of the SUPRA CAR



CAR-scFv vs zipCAR and zipFv through leucine binding = greater flexibility in antigen recognition



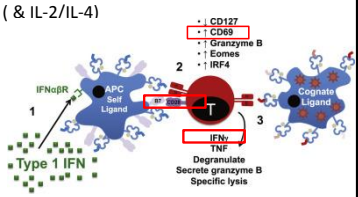
Multiple tumor antigen targetings with zipFvs

Background: T cell activation

Conventional CAR

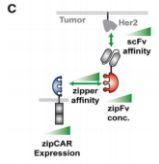
- Antigens on APCs bind to T cells
- Intracellular domains are activated (CD28, CD3)
- CD69 expression is increased
- IFN-gamma is secreted (& IL-2/IL-4)

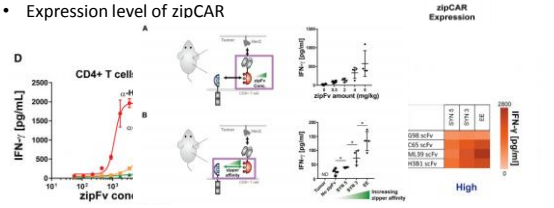
Used as marker for recognition in this paper



Design and Characterization of the SUPRA CAR

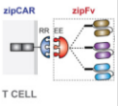
- Affinity between leucine zipper pairs
- Affinity between tumor antigen and the scFv
- Concentration of zipFv
- Expression level of zipCAR

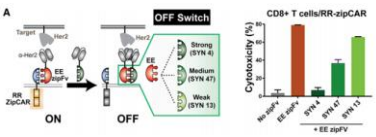


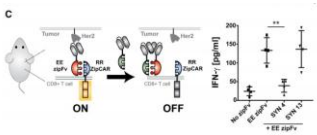


Increased safety: Off switch function

Goal: Prevent cytokine release syndrome

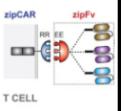


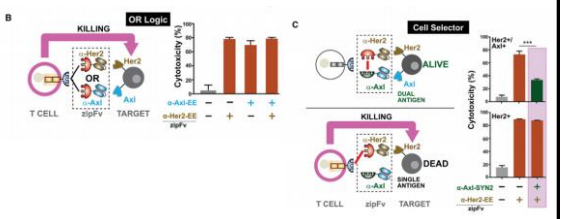




Increased safety: Combinatorial antigens targeting

Goal: combat antigen escape caused by tumor evasion b downregulating antigens & increase tumor specificity





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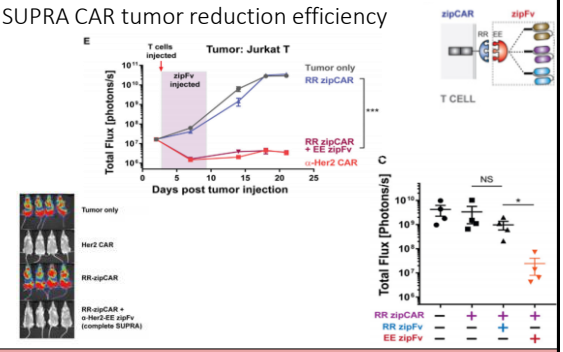
SUPRA CAR tumor reduction efficiency



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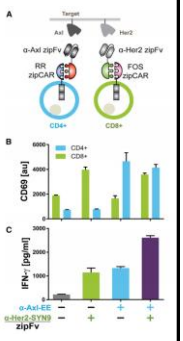
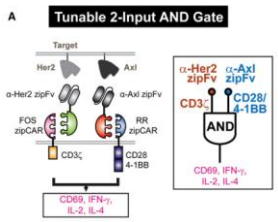
Controlling different cell types with Orthogonal SUPRA CARs

Goal: mitigate immunogenicity against synthetic leucine zippers & control different T cell subtypes





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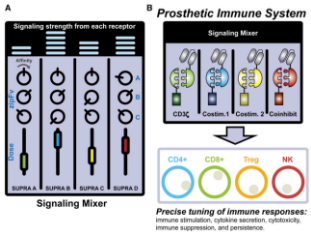
"SUPRA CAR: The swiss army knife of CAR"
"Engineering a prosthetic immune system with SUPRA CARS"



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Thank you

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Anton Omelchenko
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Dr. Ann Stock
Dr. Martin Yarmush

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Biotech Discussion 2/22/19

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Rigor & Reproducibility

- How is the QC criteria defined? Will this vary between patients?
- How rigorous are QC checks/replicates of data/clinical studies?
- Are patient cells a good source for CAR-T (as patients are sick)? Will this be reproducible between batches/patients?
  - "Allogeneic CAR T therapies have a number of potential advantages over autologous CAR T approaches. First, the cells are derived from a healthy donor, and the donor can be pre-screened for the desirable number, CD4:CD8 ratio, and phenotype of T cells."- MacLeod et al
- Is it reproducible between different labs, manufacturing sites, with different (lots) reagents used?
  - "The process is scalable and compatible with good manufacturing practices (GMP)-compliant manufacturing. With the exception of the AAV vector, the process relies on growth media, reagents, and equipment that are commonly used in the manufacture of CAR T products that are currently being evaluated in phase I and phase II clinical trials. Fortunately, because of their prominent role in gene therapy, AAV vectors have a well-established regulatory history and safety profile and are readily produced under GMP conditions." - MacLeod et al.
- How to account for variability/biological difference between patients?

Ethics and CAR-T (cell) Therapies

- How to manage toxicity (patient **safety**) in CAR-T therapies? Is the risk worth the reward?
- How well are these risks communicated? Do patients understand their consent? Can patients consent to risks that may be unknown (transplants)?
- How to ensure equal **access** to innovative CAR-T therapies?
  - EU medical insurance does not cover cell therapy (who decides coverage?)
  - Financially: 1 treatment (sufficient) of Kymriah is ~$500k
  - Geographically: Yescarta therapy only available at 50 US hospitals
- How to manage expectations/needs and growing patient **demands**?
- Should healthy patients be engineered with CAR fragment in case of disease (eNSCs/preventative medicine)?
- In the transition from autologous to allogenic therapies, who are donating the cells for other patients/company financial benefits? Are they receiving anything ($) from it?

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THE STATE UNIVERSITY OF NEW JERSEY

AMERICAN SOCIETY of GENE & CELL THERAPY

Integration of a CD19 CAR into the TCR Alpha Chain Locus Streamlines Production of Allogeneic Gene-Edited CAR T Cells

Daniel T. MacLeod, Jeyaraj Antony, Aaron J. Martin, Rachel J. Moser, Armin Hekele, Keith J. Wetzel, Audrey E. Brown, Melissa A. Triggiano, Jo Ann Hux, Christina D. Pham, Victor V. Bartsevich, Caitlin A. Turner, Janel Lape, Samantha Kirkland, Clayton W. Beard, Jeff Smith, Matthew L. Hirsch, Michael G. Nicholson, Derek Jantz, and Bruce McCreedy

Emily DiMartini
Topics in Advanced Biotechnology II
February 22, 2019

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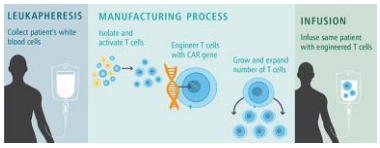
Autologous vs. Allogeneic Cell Therapy



- Allogeneic CAR T cells from healthy donors are an alternative towards an off-the-shelf therapy
  - Graft versus host disease (GvHD)
  - Initiated by endogenous T cell receptor (TCR)

Gene editing techniques can be used to eliminate expression of the endogenous TCR

2

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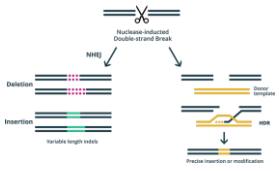
Gene Editing for Cell Therapy

- Most studies focus on lentiviral or retroviral vectors to insert CAR expression cassette into T cell genome
  - Yields semi-random integration, heterogenous expression, and insertional mutagenesis
- Exploit homology-directed repair (HDR) to insert transgene at a defined site in the genome
  - Adeno-associated viral (AAV) vectors and homing endonucleases
  - More consistent and safe product



Novel gene editing approach to target insertion of a CAR expression cassette and simultaneously knock out the native TCR via an AAV6 vector and site-specific endonuclease

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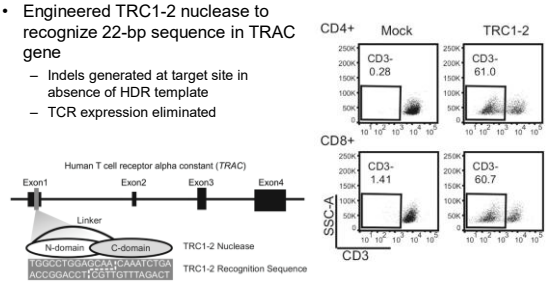
TRC1-2 Nuclease Disrupts TCR Expression

- Engineered TRC1-2 nuclease to recognize 22-bp sequence in TRAC gene
  - Indels generated at target site in absence of HDR template
  - TCR expression eliminated



TRC1-2 nuclease induces DNA breaks at TRAC locus, which knocks out TCR and prevents allo-reactivity.

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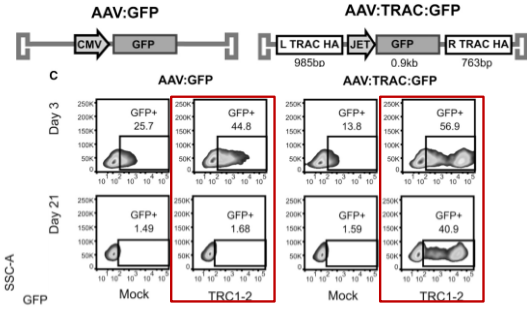
GFP Integration into TRAC Locus

- Produced AAV6 vectors carrying GFP expression cassette
  - With or without sequences homologous to the TRAC locus.



Stable GFP transgene targeted to TRAC locus via HDR

5

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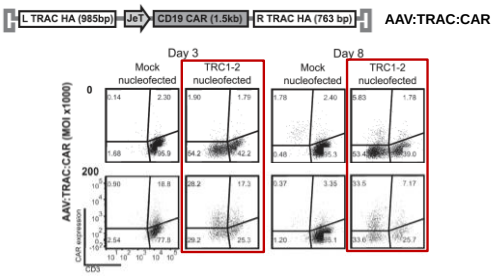
Targeted Insertion of Anti-CD19 CAR Gene

- AAV6 vector with an anti-CD19-BB-zeta CAR expression cassette flanked by TRAC homology arms
  - Multiple vector doses were evaluated by PCR and flow cytometry

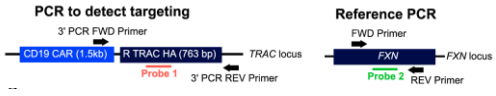


TRC1-2 nuclease can target CAR transgene insertion into TRAC locus

6

RUTGERS Targeted Insertion of Anti-CD19 CAR Gene

- Further quantified integration efficiency with digital droplet PCR
  - ddPCR assay uses two different primer sets labeled with TaqMan probe



RUTGERS Targeted Insertion of Anti-CD19 CAR Gene

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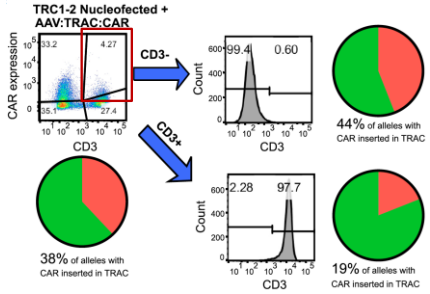
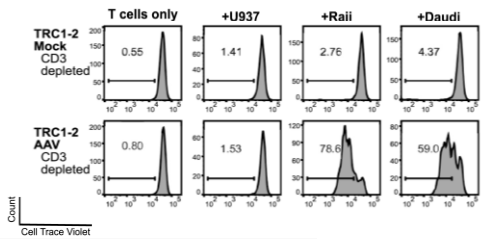


ddPCR confirms efficient targeted insertion of anti-CD19 CAR into TRAC locus, frequently with TCR knockout

RUTGERS TCR Knockout CAR T cells In Vitro

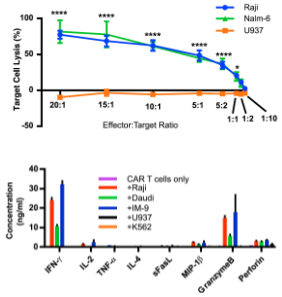
- Anti-CD19 CAR T cells were characterized for functional activity *in vitro* compared to controls
  - Proliferation in response to antigenic stimulation as marker
  - CD19$^+$ Raji or Daudi cells and CD19$^-$ U937 cells



CD3 depleted CAR T cells proliferate in the presence of CD19 antigen

RUTGERS TCR Knockout CAR T cells In Vitro

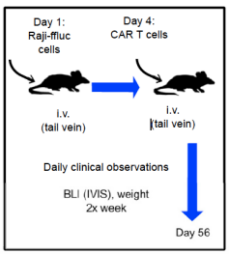
- Anti-CD19 CAR T cells were characterized for functional activity *in vitro* compared to controls
  - CD19$^+$ Raji, Daudi, IM-9 cells
  - CD19$^-$ U937 and K562 cells
  - Cytolytic activity and pro-inflammatory cytokine release to confirm functional activity



Anti-CD19 CAR$^+$ T cell activity is target specific

RUTGERS Anti-CD19 CAR T cells In Vivo

- Murine model of disseminated B cell lymphoma with i.v. injection of luciferase-expressing Raji cells
  - CD3 depleted anti-CD19 CAR T cells injected at 3 different doses
  - Controls of TCR$^-$ T cells or PBS



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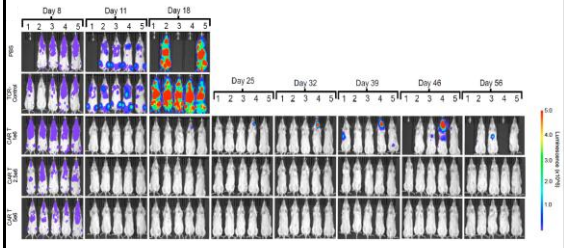

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Anti-CD19 CAR T cells In Vivo

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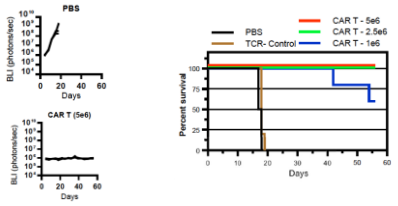

Results demonstrate potent *in vivo* clearance of CD19$^+$ tumor cells by CD3$^-$ CAR T cells

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Conclusion

- Developed streamlined method for allogeneic CAR T cell manufacturing
  - Single gene-editing step where CAR is expression cassette is targeted to the TRAC locus
  - High level of targeted gene insertion with nuclease/AAV
  - T cells can be obtained from a healthy patient, yielding more control over T cell number and phenotype
  - Scalable and compatible with good manufacturing processes

Critiques:

- Characterization of T cell allo-reactivity
- Controls and cell dosing in *in vivo* model

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Acknowledgements

- Dr. Biju Parekkadan
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- Jeremy Anderson
- Mollie Davis
- Alex Burr

NIH Biotechnology Training Fellowship (NIH T32 GM008339)

The in Biomedical Engineering: The Unknown and The Unexpected

Biotechnology Training Program
March 8, 2019

Cell Transplantation Module

Team Leader: Yoliem Miranda

Paper Reviews (2):

- Brandon Newton: Photoreceptor Transplantation
- Caroline Wood: iPSC for Retinal Pigment Epithelium

Rigor & Reproducibility: Lauren

Ethics in Research: Alison Acevedo

Maribel VaZqueZ, Sc.D.
Associate Professor
Dept of Biomedical Engineering

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OVERVIEW

Fundamental Mission of Biomedical Engineering:

" Better the world and improve Human condition through interdisciplinary and creative merging of engineering, biology and medicine."

- ***Eye Health & Age-related Disorders*** (One in Four Americans will suffer adult vision loss, NEI)
- ***BME Research in the Visual System*** (Cornea, Lens, Vitreous Humor, Choroid, Retina)
- **Ethics and Health Disparities** (Different US communities experience undue health burden)

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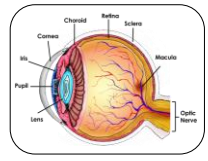
VISION IN ADULTS

- **Vision Loss** is the partial or complete loss of sight that is uncorrectable (e.g. lenses) and may occur suddenly or over a period of time.
- Americans have 1 in 4 probability of vision loss over age of 65 (AFB.com)
- 25M adults with impaired vision; A 50% increase in adults with vision loss is expected by 2050 (NEI)
- Vision loss is complex and can occur via different components of the eye
- Growing focus on epigenetics and age-related vision loss

Common Ocular Illnesses in USA

- Trauma (Injury)
- Diabetic Retinopathy
- Cataracts
- Glaucoma
- Macular Degeneration
- Genetic Disorders



- Global Health Initiative to eliminate preventable vision loss by 2020

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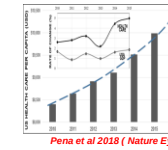
RIGHT TO SIGHT 2020

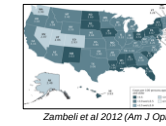
- Vision loss is the primary fear of working Americans *and* primary cause of US worker injury and disability (*US Dept. of Labor, 2012*)
- Increasing US health care costs are strongly driven by age-related disorders
- **Health Disparities (HD)** are differences in incidence, burden and treatment of illness based on non-medical factors e.g. socio-economic status, gender, geography, ethnicity, age, employment…
- Large HD in adult vision loss in USA and worldwide; (Among Current WHO Global Health Challenges)



Pena et al 2018 (Nature Eye)

Vision Disparities across USA

Dysfunctions	Affected Groups
Cataracts	Hispanics, Females
Glaucoma	Asians, Females
Macular Degeneration	White, Males
Diabetic Retinopathy	Blacks, Females



Zambeli et al 2012 (Am J Opht)

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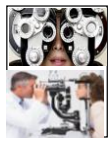
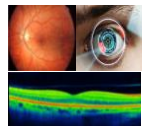
THE EYE & TECHNOLOGY



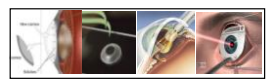


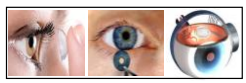
Diagnostics & Imaging

- Optometry and lenses
- Tonometry test
- Fundus photography
- Optical Coherence Tomography
- Retinal Scans / Iris Scans

Lasers & Biomaterials

- Excimer lasers (LASIK)
- Keratectomy & artificial cornea
- Lens biomaterials





Prosthetics & Implants

- Contact lenses
- Iris implants
- Retinal prostheses

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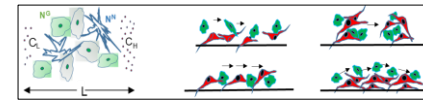
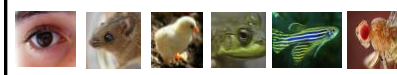
REGENERATIVE THERAPIES

- Regenerative therapies seek to recapitulate developmental processes with replacement stem-like cells (STLCs): Cell Fate, behavior and response
- Collective and integrated responses of STLCs are fundamental to development during formation of neural tube, forebrain, optic cup
- Extraordinary collective behaviors develop the cell-to-cell connectivity for vision



- Pathways guiding eye development are well-conserved across species

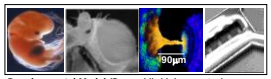


- The **RETINA** converts photonic energy into vision

CURRENT PROJECTS

Our lab researchers develop experimental models to quantitatively examine collective behaviors and responses of stem-like cells (STLCs) in the retina.

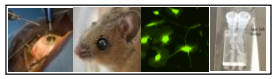
RETINOGENESIS: Genetic developmental models to evaluate contribution of cellular microenvironment and genotype to STLC migration in Optic Stalk.



Developmental Model *(Drosophila Melanogaster)*

- Simple Model: 800 Ommatidia
- Fully-mapped Genomics/Proteomics
- Ex/In vivo imaging (No IACUC!)
- Microscale optic structures (larvae)

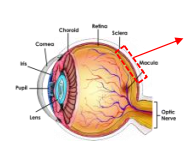
REGENERATION: Experimental models to predict in vivo STLC integration via cell responses to cues from adult tissue environment.

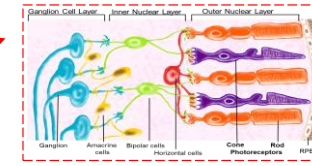


Degeneration Model *(Light-induced)*

- Mammalian retina (IACUC Needed)
- Transplant P6 STLCs to adult
- Focus on day vision
- Cone photoreceptor progenitors

ADULT HUMAN RETINA

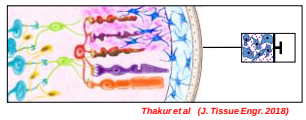




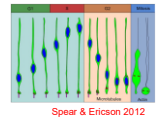
- Photoreceptors synaptically interconnect with neurons to enable vision
- Damaged photoreceptors do NOT regenerate → permanent vision loss
- STLC cells can be introduced to replace degenerated cells
- Transplanted in micrometer proximity of desired synaptic targets
- Focus has been on specialized STLCs and their biomaterial delivery; Modest and mixed results (animal models and Humans)
- Few studies have explored effects of microenvironment on STLC behavior, despite known differences in embryonic, post-natal and adult tissues

DAMAGED RETINA

Can we stimulate transplantable STLCs to migrate? (**IKNM versus Chemotaxis**)

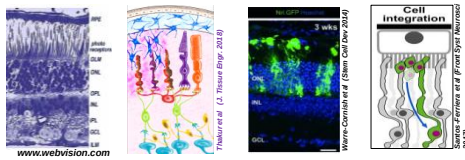


Thakur et al (J. Tissue Engr. 2018)



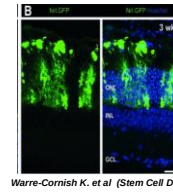
Spear & Ericson 2012

- Idealized 4-Step Process: Delivery, Migration, Extension & Synapse
- Previous studies have demonstrated ~25% of STLC integration

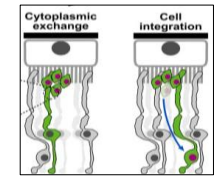


DAMAGED RETINA

- Recent studies have illustrated STLC transplantation *can yield new synapses OR create material transfer to native Photoreceptors*
- Microenvironment impacts synaptogenesis (wild-type model versus degeneration or disease), but effects on STLC migratory processes remain only partially explored



Warre-Cornish K. et al (Stem Cell Dev 2014)



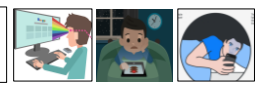
Santos-Ferreira et al (Front Syst Neurosci 2017)

- Modeling adult microenvironments is critical to regenerative strategies:
  - Inherited retinal diseases can trigger degeneration in adulthood
  - Epigenetic and age-related changes in retinal microenvironments

REGENERATION

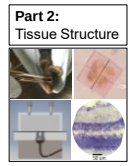
GOAL: Develop experimental model to predict extent of in vivo STLC integration by evaluating cell responses within microenvironments of degenerated retina.

Light-Damage Model (λ_{BLUE}=465nm)
- Workplace injuries and disabilities
- Macular degeneration

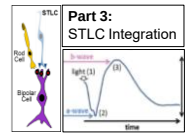


Part 1: Cell Microenvironment

Part 2: Tissue Structure



Part 3: STLC Integration



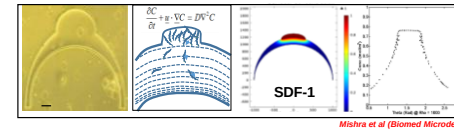
KEY RETINAL LIGANDS

- We used Bioinformatics (with IPA Scientific®) to explore Light-Damaged Mouse Model ($p<10^{-4}$)
- In vivo concentrations, gradients remain unknown
- Over 10^3 genes for photoreceptor surface receptors and > 10^3 known injury ligands
- Revealed only **5** combinatory ligand::receptor pairs

GDNF :: RET
SDF1 :: CXCR4
EGF :: EGFR
VEGFA:: VEGFR2
PDGF :: PDGFR

Unachukwu et al (Sci Rep 2017)

- Develop µRetina system to generate controlled concentration profiles of targeted ligands at geometrical scale of mammalian retina



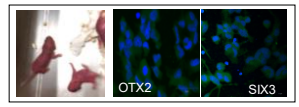
Mishra et al (Biomed Microdev 2015)

- Can we stimulate populations of transplantable STLCs to migrate?

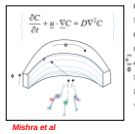
THE μRETINA SYSTEM

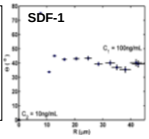
- **Mouse Model:**

C57BL/6J Host Adult Mouse (Wild-type); I_{BL} =4mW/m^2
Crx GFP$^+$ Rod and Cone Precursors (P4)



- **Highly sensitive Chemotaxis:**





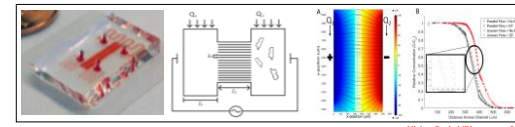
Mishra et al (Biomed Microdev 2015)

- Examine migration to EGF, PDF, VEGF, SDF-1
- Directed motility to SDF-1 seen in μRetina in r, θ and ϕ directions
- Cells migrated singly, collectively
- Motility Index as per literature ~25%
- FACS isolation of motile phenotype requires 10^5-10^6 cells

- *Can we use electric fields to increase migration capacity?*

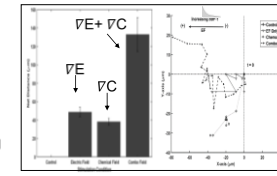
GALVANO-CHEMOTAXIS

Can we use Electric Fields to increase motility? (MμN System)



McCutcheon S. et al (Biomed Microdevices 2017) — Mishra S. et al (Biosensors 2017)

- Cells and matrix loaded in macro-sized channels, cells seeded within micro-scaled chambers; Nanoarray provides separation and controlled transport
- Chemical field (∇C) not affected by superimposed electric field (∇E)
- Directionality and net distances traveled increased more than 3 times using combo fields than either alone

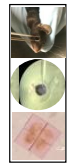


Mishra et al (Biosensors 2017)

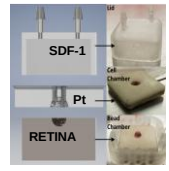
RETINAL EXPLANTS

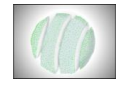
STLC with retinal ECM and communication with native neuronal groups

i. Ex Vivo (Retinal Explant)
- Traditional, technique-driven
- Micro-macro system for retina
- Galvano-Chemotactic Injection
- Enables ∇ E and ∇C fields
- Control testing and alginate beads



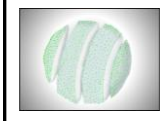
ii. Ex Vivo (EVES System)
- Ex Vivo Eye Facsimile System
- Micro-macro system for whole eye
- Galvano-Chemotactic Injection
- Enables ∇ E and ∇C fields
- Control testing and alginate beads



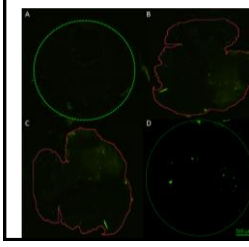


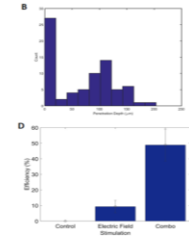
EVES TESTING SYSTEM

EVES System enable testing of galvano-chemotactic injection



- Fluorescent cells migrate into alginate facsimile of eye
- The penetration depth and infiltration efficiency is estimated per slice
- The galvano-chemotactic injection increased # of cells that infiltrate into eye facsimile by a factor of 5.

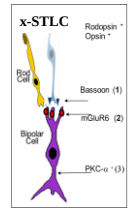




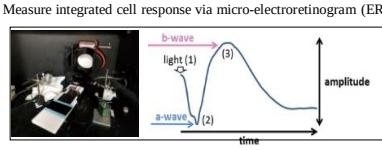
ELECTRO-RETINOGRAMS

Part 3: Synaptic Integration across Cells

- STLC positioning and communication with secondary neurons (Bipolar cells)
  - Bassoon (Pre-synaptic)
  - mGluR6 (Post-synaptic)
  - PKC-a$^+$ (ON Bipolars)



- Measure integrated cell response via micro-electroretinogram (ERG)



FUTURE DIRECTIONS

National Eye Institute funding only for models that mimic Human:
- Must have macular zone and be cone-rich (No rodents!)
- Suggested Porcine ($$) or Macaque ($$$ + ethical)
- MV Strategy: Human cadaver explants (Eye Bank)

THANK YOU FOR YOUR ATTENTION!

Dr. Sean McCutcheon
Dr. Shawn Mishra
Mr. Juan Pena
Ms. Caroline Pena
Mr. Kameron Starr
Ms. Tanya Singh
Ms. Stephanie Zhang

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THE STATE UNIVERSITY OF NEW JERSEY

Protective Effects of Human iPS-Derived Retinal Pigment Epithelium Cell Transplantation in the Retinal Dystrophic Rat

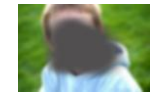
Carr A-J, Vugler AA, Hikita ST, Lawrence JM, Gias C, et al.

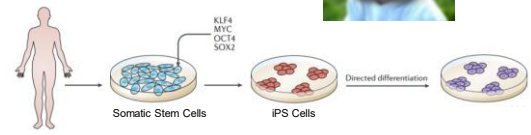
Caroline Wood
Biotechnology Training Program
March 8, 2019

Stem Cell Therapy for Retinal Disease

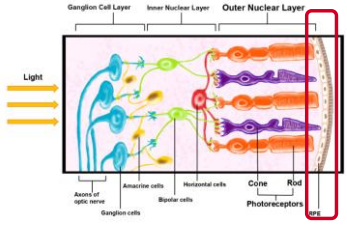
- **Age Related Macular Degeneration**
  - Rod and cone photoreceptor degeneration leads to blindness

- **Stem Cell Therapies**
  - Induced Pluripotent Stem Cells



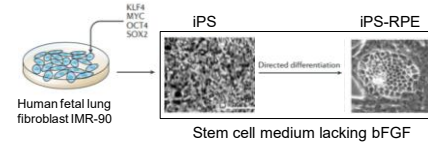


Retinal Pigment Epithelium (RPE)



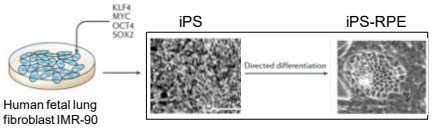
- Critical for photoreceptor function and survival
  - Phagocytose debris/photoreceptor outer segments
  - Transport molecules and metabolic waste
- Accessible target for cell therapy

Overview of Paper



Stem cell medium lacking bFGF

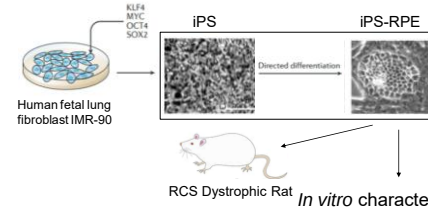
Overview of Paper



In vitro characterization and functionality

Western blot,
Quantitative PCR,
Electron Microscopy,
Phagocytosis assay

Overview of Paper



In vitro characterization and functionality

Western blot,
Quantitative PCR,
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Phagocytosis assay

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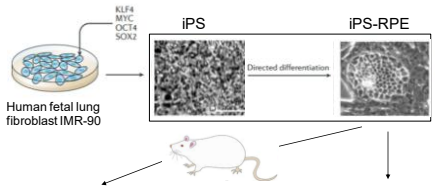
Overview of Paper



In vivo functionality after transplantation

Optokinetic Testing
C-Fos Staining
Immunohistochemistry

In vitro characterization and functionality

Western blot,
Quantitative PCR,
Electron Microscopy,
Phagocytosis assay

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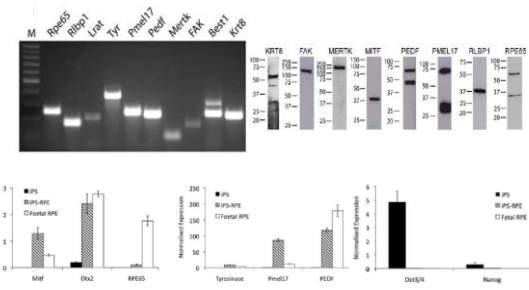
iPS-RPE Express RPE Markers



Similar protein expression profile to foetal RPE

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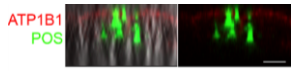
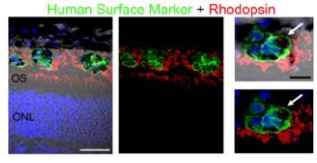
iPS-RPE Are Capable of Phagocytosis



In vitro



In vivo
(8 days post transplantation)

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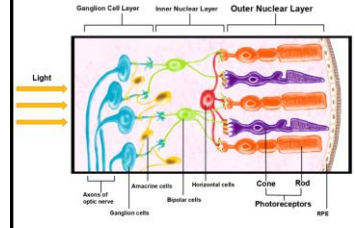
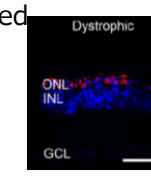
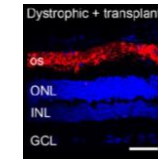
Retinal Structure Preserved







(13 weeks post implantation)

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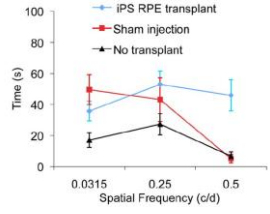
Retinal Function is Preserved

Optokinetic Testing



(13 weeks post implantation)

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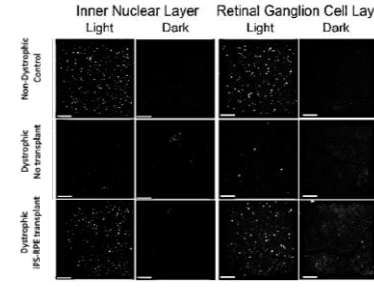
Functional Retinal Neuronal Circuitry



Light induced c-Fos response
(13 weeks post implantation)

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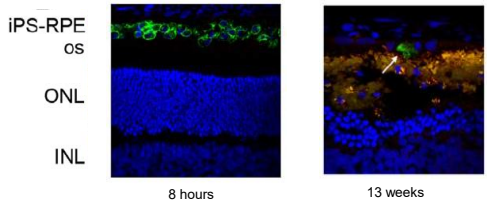
iPS-RPE Rejection



Few transplanted cells remain 13 weeks after transplantation

HESC-derived RPE have been shown to survive up to 30 weeks

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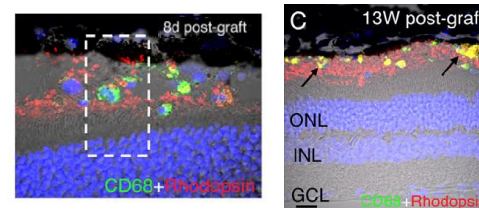
Secondary Protective Host Response



- Macrophage/Microglial infiltration and clearance of debris may contribute to photoreceptor survival
- iPS-RPE may have secreted neurotrophic growth factors

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Future Considerations for Therapy

- The host inflammatory/immune response to the xenograft and iPS immunogenic profile should be studied
- Patient specific iPS therapies may reduce problems associated with immune rejection
- Combination of iPS with gene therapy
- iPS cell reprogramming risks integrating stem cell genes into the genome

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Acknowledgements

Biotechnology Training Program
NIH T32 GM008339

- Dr. Maribel Vasquez
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- Alison Acevedo
- Lauren Timmins
- Brandon Newton

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Questions?

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Migration, Integration and Maturation of Photoreceptor Precursors Following Transplantation in the Mouse Retina

Katherine Warre-Cornish,[1] Amanda C. Barber,[1] Jane C. Sowden,[2] Robin R. Ali,[1,3] and Rachael A. Pearson[1]

Brandon Newton
Biotechnology Training Program
March 8, 2019

Characterized Rod Transplantation

Related Macular Degeneration
- Age 1 in 10 people over 60.
- An oval yellowish area near the center of the retina. Region of keenest vision.

Literature shows transplanted photoreceptors can be incorporated and maintained in non-neurogenic adult retinal environments.

First study that examines the period immediately following transplantation (48 h) until 6 weeks post transplantation in order to determine the time course of cell integration.

2

Parameters for Evaluation

Quantification of proportions of transplanted rods

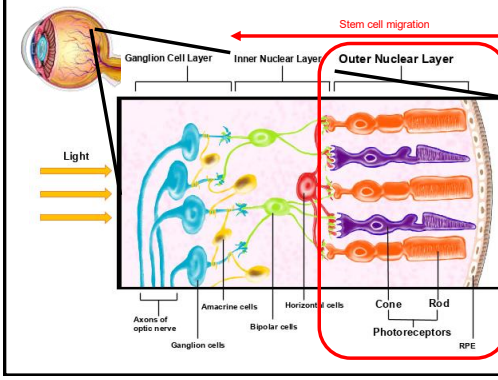
Parameter	Measurement
Study of Morphologies	Architectural Integrity
Alpha Transducin Expression	Rate of Migration
Optomotor Response	Functionality
Ribeye Expression	Network Formation
Macrophage Grading	Level of Inflammation
Nuclear Architecture Analysis	Maturation

C57Bl/6J, Nrl.GFP (+/+) mice

NRL = Neural retina-specific leucine zipper protein
GFP = Green Fluorescent Protein

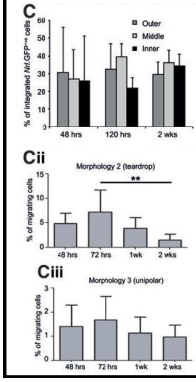
3

Anatomy of the Eye

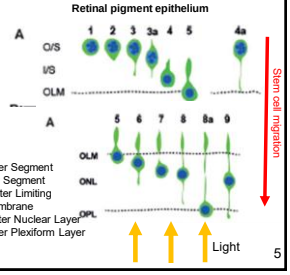


4

Morphologies in the Interphotoreceptor Matrix



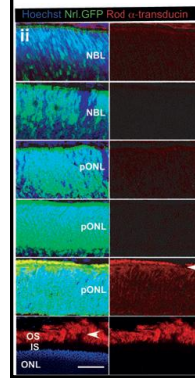
- Donor cells migration from initial cell mass bolus through the interphotoreceptor matrix
- Morphology 4a - donors that fail to cross the outer limiting membrane (OLM) into the recipient outer nuclear layer (ONL).



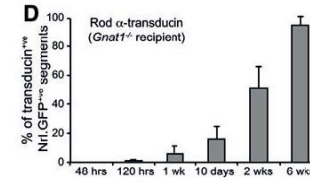
O/S = Outer Segment
I/S = Inner Segment
OLM = Outer Limiting Membrane
ONL = Outer Nuclear Layer
OPL= Outer Plexiform Layer

5

Rod Alpha-transducing During Development



- Mature Retina universally rod alpha transducing negative.
- Rod alpha transducing may represent a market for defining the end of the integration.
- Expression of rod a-transducing was delayed relative to normal development but increased over time post transplantation.



NB = Neuroblastic Layer
ONL = Outer Nuclear Layer

6

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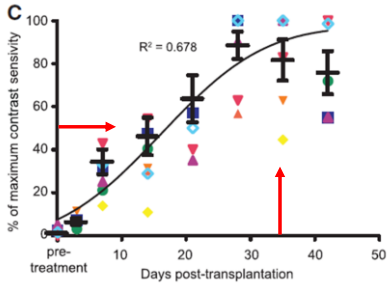
Optomotor Response

Separate study used to study the head tracking behavior in Gnat1 -/- mice (stationary night blindness) in response to movement



7

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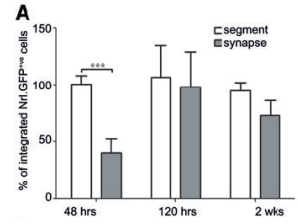
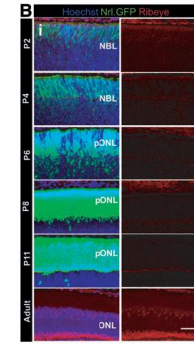
The Importance of Ribeye in Cell Development

Ribeye is a protein used for building ribbon synapses to transmit sensory signals.





8

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Gaging Inflammation with Macrophage Grading

15% of transplanted retina showed signs of inflammation and were associated with graft failure.

When examined at earlier time points:

- 72 hours (n =14): 50% apoptosis of cells
- 120 hours (n =27): 70% apoptosis of cells.

Transplants associated with a transient inflammatory reaction that peaks at 120 h and resolves in most cases by 2-3 weeks post transplantation.

9

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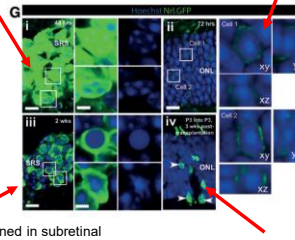
Nuclear Architecture Analysis

Multichromocenter nuclear architecture in donor cells in the subretinal space 48 hours after injection.

Condensed single chromocenter nuclear architecture in cells after 72 hours.



Cells that remained in subretinal space mature architecture by 2 weeks post transplantation.

Immature nuclear architecture 3 weeks post-transplantation.

10

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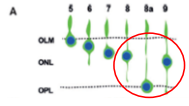
Updates to Study

Since 2014 there are new studies which imply that the cell movement during transplantation characterized in this paper may not be occurring as described.

- Cytoplasmic exchange: Cellular material is transferred from layer to layer without the actual migration of the cell body.
- Paper did not track the dose response of vision.
- Also the apoptotic signaling present during transplantation may give confounding results.



11

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NIH T32 GM008339

- Dr. Maribel Vazquez
- Yoliem Miranda Alarcón
- Alison Acevedo
- Lauren Timmins
- Caroline Wood

- Dr. Joseph Freeman
- Dr. Yarmush

12

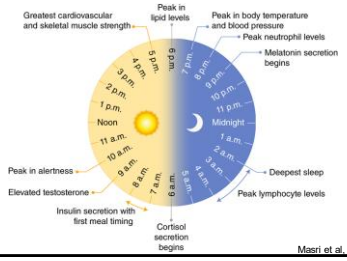
BMAL1 knockout macaque monkeys display reduced sleep and psychiatric disorders

Peiyuan Qiu, Jian Jiang, Zhen Liu, Yijun Cai, Tao Huang, Yan Wang, Qiming Liu, Yanhong Nie, Fang Liu, Jiumu Cheng, Qing Li, Yun-Chi Tang, Mu-ming Poo, Qiang Sun, Hung-Chun Chang

National Science Review (2019)

Skylar Chuang
Biotechnology Training Program Spring, 2019

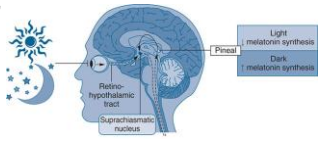
Background: Circadian Rhythm



Masri et al, *Nat Med* **24**, 1795-1803 (2018)

Circadian rhythm is largely controlled by BMAL1

- The master circadian clock is located within hypothalamic suprachiasmatic nucleus (SCN), but key circadian genes are well expressed in peripheral tissue, thereby contributing to periodicity for many organs
- BMAL1 (Brain and Muscle ARNT-Like 1) is a basic helix loop helix transcription factor that plays a key role in generating circadian rhythm
- Previous studies showed that *Bmal1* knockout mice had:
  - Loss of circadian rhythm
  - Infertility
  - Defective glucose metabolism
  - Idiopathic calcification of limb joints
- However, rodent models have limited phenotypes especially in regard to psychiatric disorders



Molina, P. E. (2010). *Endocrine physiology*. New York: McGraw-Hill Medical.

Rationale for using non-human primates

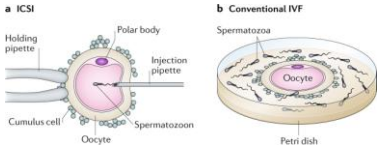
- A suitable model should display physiological features similar to humans
  - Non human primates exhibit more complex behaviors compared to mice
    - Dynamic social structures
    - Deep homology in brain circuitry mediating social behavior
  - Nocturnal rodents do not recapitulate the diurnal behavior of primates
    - Distinct phases of gene expression exist between the transcriptome atlas of baboons vs mice (Mure, *Science*, 2018)

Parameters to evaluate BMAL1-KO monkeys

- Several experiments were performed to evaluate the effects of BMAL1 knockout on:
  - Locomotor activity
  - Blood hormone levels
  - Sleep states
  - Behavior
  - Transcriptome

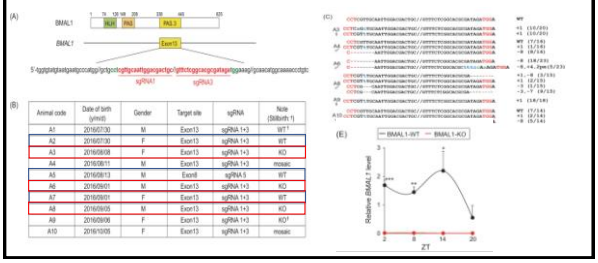
Model: BMAL1 knockout macaque monkeys

- CRISPR/Cas9 gene editing was performed to knockout of BMAL1 in the zygote obtained via intracytoplasmic sperm injection:
  - Total of 88 edited embryos
  - 31 females used as surrogates
  - 10 pregnancies; 8 healthy live births



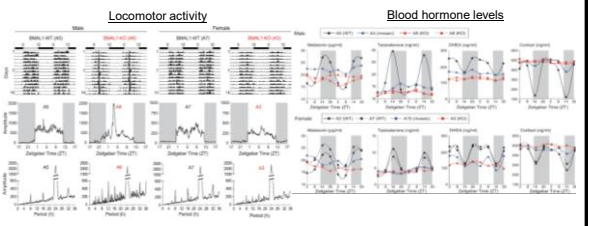
Esteves et al, *Nat Rev Urol*, **15**,535-562 (2018)

Knockout of BMAL1 using CRISPR/Cas9 gene editing



Animal code	Date of birth (y/m/d)	Gender	Target site	sgRNA	Note (Stillbirth ?)
A1	2016/07/30	M	Exon13	sgRNA 1+3	WT
A2	2016/07/30	F	Exon13	sgRNA 1+3	WT
A3	2016/08/08	F	Exon13	sgRNA 1+3	KO
A4	2016/08/11	M	Exon13	sgRNA 1+3	mosaic
A5	2016/08/13	M	Exon8	sgRNA 5	WT
A6	2016/09/01	M	Exon13	sgRNA 1+3	KO
A7	2016/09/01	F	Exon13	sgRNA 1+3	WT
A8	2016/09/05	M	Exon13	sgRNA 1+3	KO
A9	2016/09/06	F	Exon13	sgRNA 1+3	KO
A10	2016/10/05	F	Exon13	sgRNA 1+3	mosaic

Activity and hormone levels in BMAL-KO monkeys

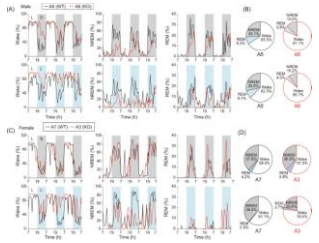


Altered sleep patterns in the BMAL-KO monkeys

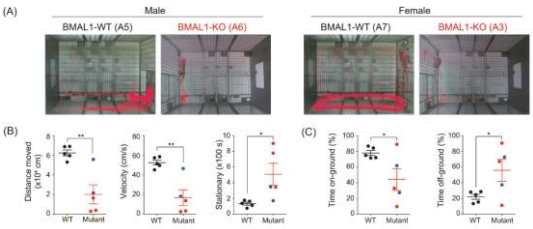
REM: Rapid eye movement

NREM: Non-REM

Image source: Verywellhealth.org

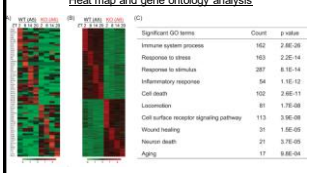


Depression related behavior in BMAL1-KO monkeys



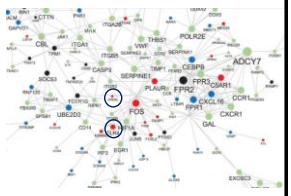
Transcriptome analysis of BMAL1 knockout monkeys

Heat map and gene ontology analysis



Significant GO terms	Count	p value
Immune system process	162	2.8E-26
Response to stress	163	2.2E-14
Response to stimulus	287	8.1E-14
Inflammatory response	54	1.1E-12
Cell death	102	2.6E-11
Locomotion	81	1.7E-08
Cell surface receptor signaling pathway	113	3.9E-08
Wound healing	31	1.5E-05
Neuron death	21	3.7E-05
Aging	17	9.8E-04

Functional network of



Summary

- ❑ BMAL1 knockout monkeys are models that
  - BMAL1 has a critical role in controlling circadian clock in most tissues, therefore its knockout has minimal redundancy
  - They exhibit phenotypes such as premature aging, arrhythmicity, sleep and metabolic disorders
  - BMAL1 knockout results in phenotypes that resemble psychiatric disorders

Limitations

- The control WT monkeys used in these experiments also underwent gene editing, so there might be potential off targeting effects that were not accounted for.

Animal code	Date of birth (y/m/d)	Gender	Target site	sgRNA	Note (Stillbirth:1)
A1	2016/07/30	M	Exon13	sgRNA 1+3	WT[1]
A2	2016/07/30	F	Exon13	sgRNA 1+3	WT
A3	2016/08/08	F	Exon13	sgRNA 1+3	KO
A4	2016/08/11	M	Exon13	sgRNA 1+3	mosaic
A5	2016/08/13	M	Exon6	sgRNA 5	WT
A6	2016/09/01	M	Exon13	sgRNA 1+3	KO
A7	2016/09/01	F	Exon13	sgRNA 1+3	WT
A8	2016/09/05	M	Exon13	sgRNA 1+3	KO
A9	2016/09/06	F	Exon13	sgRNA 1+3	KO?
A10	2016/10/05	F	Exon13	sgRNA 1+3	mosaic

- Additional WT control monkeys with similar attributes but not having undergone gene editing will be

Acknowledgement

- Dr. ZhiPing Pang
- Group: Christopher Rathnam, Andrew Boreland, Josh Leipheimer, William Pfaff
- Biotechnology Training Program

RUTGERS

Assembly of functionally integrated human forebrain spheroids

Fikri Birey, Jimena Anderson, Christopher D. Makinson, Saiful Islam, We wei, Nina Huber, H. Christina Fan, Kimberly R. Cordes Metzler, Georgia Panagiotakos, Nicholas Thom, Nancy A. O'Rourke, Lars M. Steinmetz, Jonathan A. Bernstein, Joachim Hallmayer, John R. Huguenard, Sergiu P. Pasca

nature

Presented by Andrew J Boreland
Pang Lab and Jiang Lab
April 5th, 2019
Topics in Biotechnology II

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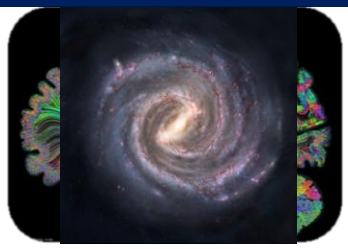
Our Brain



86 Billion neurons with 1,000's synapses/neuron

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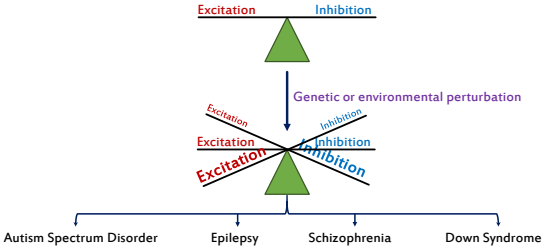
What is the problem?



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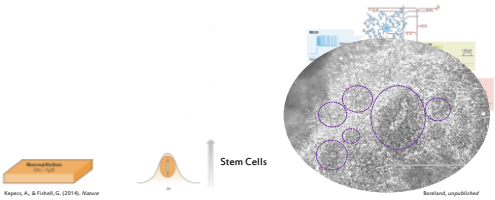
Brain Development Background



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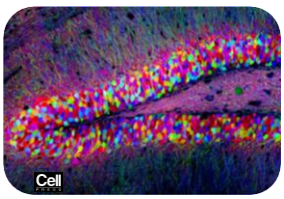
Why do we need organoids?



Pasca Lab Stanford

"A critical challenge in understanding the intricate programs underlying development, assembly and dysfunction of the human brain is the lack of direct access to intact, functioning human brain tissue for detailed investigation by imaging, recording, and stimulation".
- Sergiu P. Pasca

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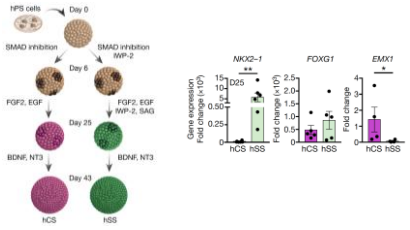
Can we make subdomain-specific forebrain organoids?



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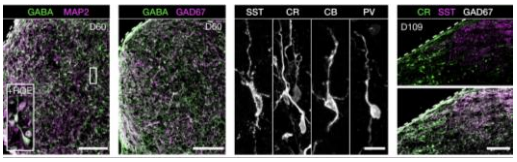
Do we see markers for interneurons in subpallium organoids?



Interneurons express appropriate markers and subtypes

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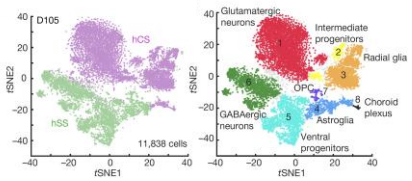
Do we see differential cell clustering between hSS and hCS by single-cell seq?



hCS and HSS cluster separately with distinct cell types

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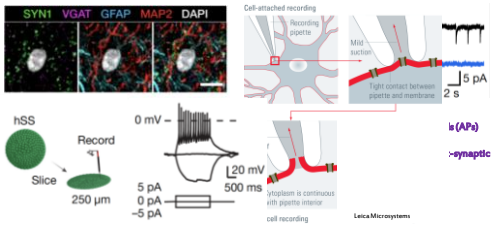
We see appropriate markers and cell clustering, but are these organoids functional?



Synaptic puncta and electrophysiology suggest functionality

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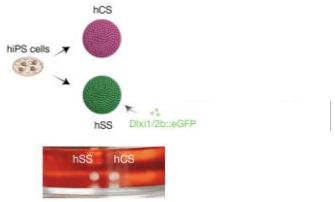
Can we develop a model for migration of interneurons into the cerebral cortex?



Fuse a subpallium organoid to a cortical organoid

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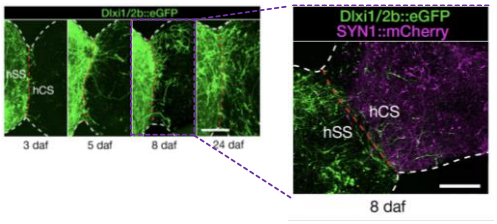
Do we see interneuron migration in these fused organoids?



Interneurons migrate unidirectionally from hSS to hCS

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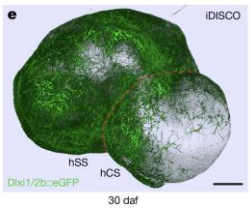
Do we see interneuron migration in these fused organoids?

Optical clearing:
Removes lipids

iDISCO



Interneurons migrate unidirectionally from hSS to hCS

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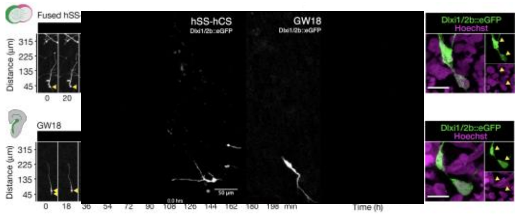
Can we track individual interneurons and characterize their movement?



[illegible]

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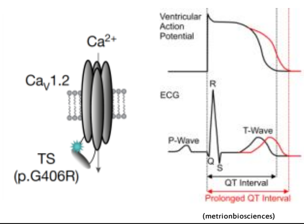
Can we use these fused organoids to model Timothy syndrome?

GOF mutation in LTCC subunit-encoding CACNA1C gene leads to Timothy syndrome (TS)



5 Control (3 female; 2 male)

3 TS Patients (1 female; 2 male)

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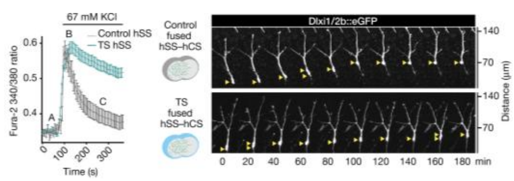
Do TS interneurons show any deficits?



Saltation frequency + saltation length and speed = less efficient migration

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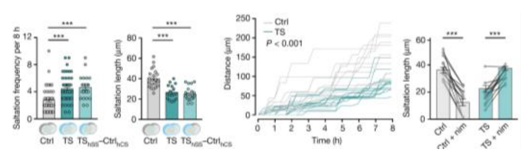
Do TS interneurons show any deficits?



Saltation frequency + saltation length and speed = less efficient migration

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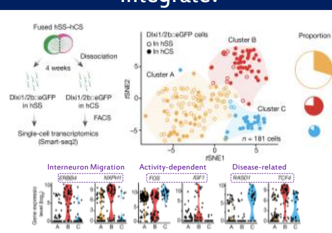
Yes the interneurons migrate, but do they integrate?



Interneurons that migrated into hCS show distinct transcriptional profile

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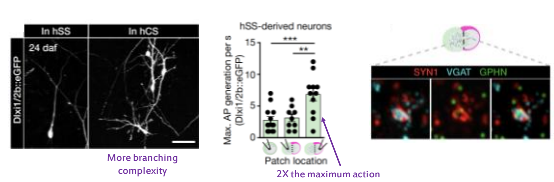
Yes the interneurons migrate, but do they integrate?



More branching complexity

2X the maximum action potential generation rate

Migrated interneurons have more complex morphology and increased activity

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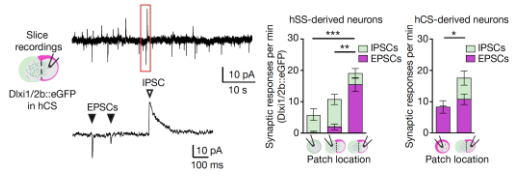
Yes the interneurons migrate, but do they integrate?



Interneurons migrate and integrate into hCS to form functional circuits

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Summary

Human forebrain organoids assembled in a dish and successfully model interneuron migration and integration

- iPS cell lines made from fibroblasts to create organoids representing two different brains regions of the human forebrain
  - 1) A cortical organoid containing excitatory neurons
  - 2) A deeper forebrain organoid containing inhibitory neurons
- Fused these organoids and found that inhibitory interneurons migrate from deep forebrain organoid into the cortical organoid and form functional connections with resident excitatory neurons
- This system recapitulates migration of fetal interneurons and their subsequent maturation and integration into cortical circuits

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Summary

Human forebrain assembloid can model aspects of Timothy Syndrome

- Mutated calcium channels in these patients interneurons leading to defective cyclic migration patterns
  - Cells moved more frequently but less efficiently, resulting in slower migration
- This migration defect was rescued by addition of chemicals that reduce the activity of the mutated calcium channels

The Septoid

Acknowledgements

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Chris Rathnam

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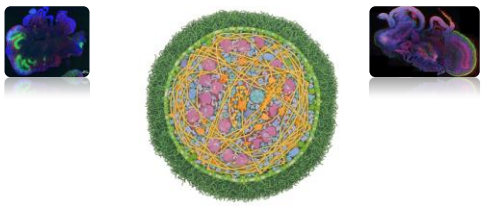
Questions?



Activation of the Nrf2 Cell Defense Pathway by Ancient Foods: Disease Prevention by Important Molecules and Microbes Lost from the Modern Western Diet

Donald R. Senger, Dan Li, Shou-Ching Jaminet, Shugeng Cao

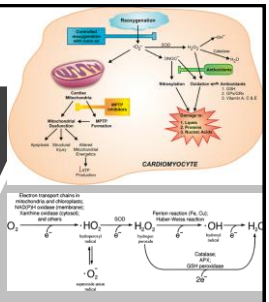
Part 1

Jeffrey Luo

1

Reactive Oxygen Species

- Reactive oxygen species (ROS) ubiquitous in aerobic environments
- Cancer, dementia, diabetes, aging

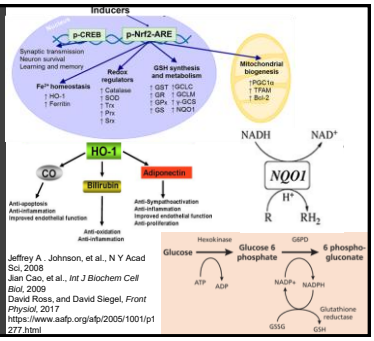


Izumi C. Mori & Julian I. Schroeder. Plant Physiol 2004
Richdeep S. Gill, et al., Can J Physiol Pharmacol, 2012
Jeffrey A . Johnson, et al., N Y Acad Sci, 2008

2

NRF2 Pathway

- Nrf2-ARE (NF-E2-related factor 2-antioxidant responsive element)
- Regulates >1% of genome
  - Heme oxygenase-1 (HO-1)
  - Glucose 6 phosphate dehydrogenase (G6PD)
  - NAD(P)H quinone dehydroxygenase (NQO-1)

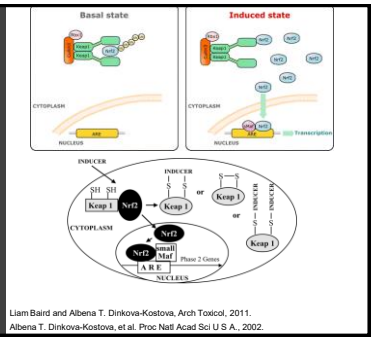


Jeffrey A . Johnson, et al., N Y Acad Sci, 2008
Jian Cao, et al., *Int J Biochem Cell Biol*, 2009
David Ross, and David Siegel, *Front Physiol*, 2017
https://www.aafp.org/afp/2005/1001/p1277.html

3

NRF2 Regulation

- Nrf2-Keap1 act as "redox" sensor
  - Absence of oxidative stress
    - $t_{½, Nrf2}$: 7-15 minutes
  - Presence of oxidative stresses
    - $t_{½, Nrf2}$: 30-100 minutes
- Synergistic effect between ROS and inducers

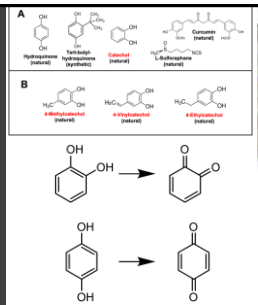


Liam Baird and Albena T. Dinkova-Kostova, Arch Toxicol, 2011.
Albena T. Dinkova-Kostova, et al. Proc Natl Acad Sci U S A., 2002.

4

Structure of Nrf2 Inducers

- Interacts with redox-active cysteine/cystine residues
- Paper oriented towards catechols



• L-sulforaphane

• 4-VC

https://en.wikipedia.org/wiki/Wine
https://www.simplyrecipes.com/recipes/roasted_cauliflower/

5

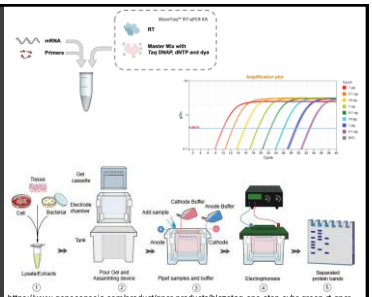
Central Question

- Can alkyl catechols activate Nrf2-ARE pathway?

6

Important Techniques

- RT-qPCR
  - Assess transcription
- Western Blot
  - Assess post-translational fate

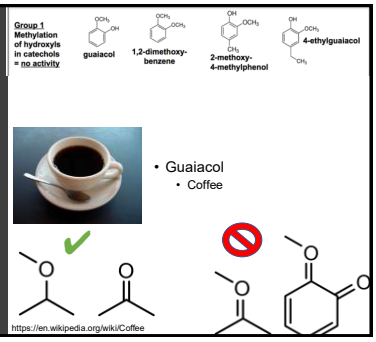


https://www.genecopoeia.com/product/qpcr-products/blazetaq-one-step-sybr-green-rt-qpcr-kit/
https://www.neb.com/luna/optimize-rt-qpcr
https://www.creative-diagnostics.com/Sample-Gel-Preparation.htm

7

Non-Functional Catechols

- Oxygen generally forms 2 bonds



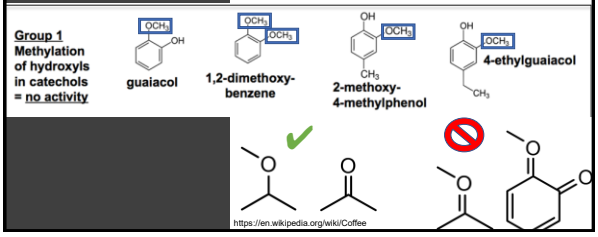
- Guaiacol
  - Coffee

https://en.wikipedia.org/wiki/Coffee

7

Non-Functional Catechols

- Oxygen generally forms 2 bonds

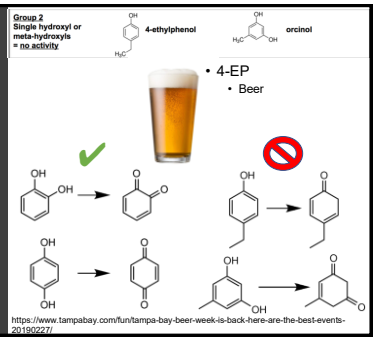


https://en.wikipedia.org/wiki/Coffee

8

Non-Functional Catechols

- Loss of aromaticity and resonance



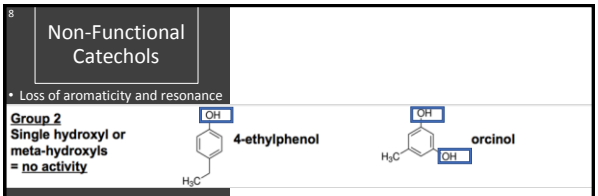
- 4-EP
  - Beer

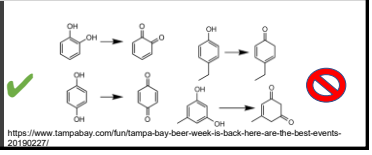
https://www.tampabay.com/fun/tampa-bay-beer-week-is-back-here-are-the-best-events-20190227/

8

Non-Functional Catechols

- Loss of aromaticity and resonance



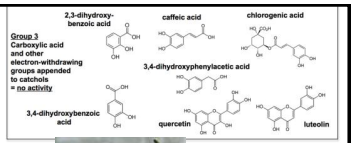


https://www.tampabay.com/fun/tampa-bay-beer-week-is-back-here-are-the-best-events-20190227/

9

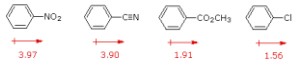
Non-Functional Catechols

- Implications to reaction rate for oxidation



- Chlorogenic acid

Deactivating Substituents

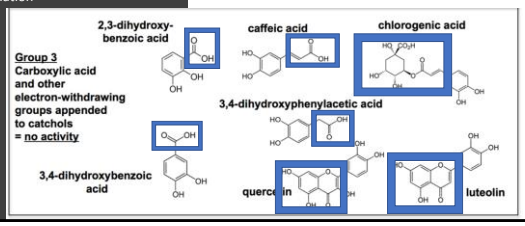


https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/chapt15.htm
https://eggplantmail.com/

9

Non-Functional Catechols

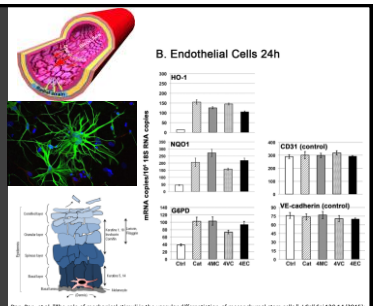
- Implications to reaction rate for oxidation



10

Nrf2 Pathway Gene Expression

- Endothelial cells, astrocytes, and keratinocytes
- Heme oxygenase-1 (HO1)
- NAD(P)H quinone dehydroxygenase (NQO1)
- Glucose 6 phosphate dehydrogenase (G6PDH)
  - p-values < 0.0005

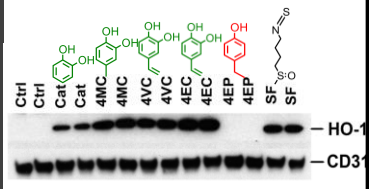


Dan, Pan, et al. "The role of mechanical stimuli in the vascular differentiation of mesenchymal stem cells." *J Cell Sci* 128.14 (2015): 2415-2422.
https://www.psypost.org/2017/11/brain-astrocytes-linked-alzheimers-disease-50221
D'Orazio, John, et al. "UV radiation and the skin." *International journal of molecular sciences* 14.6 (2013): 12222-12248.

11

Nrf2 Pathway Proteins

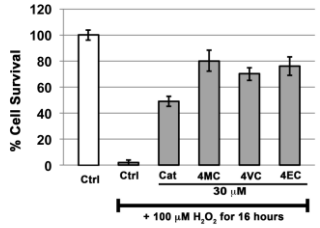
- Endothelial cells after 24 hour



12

Enhanced Cell Survival

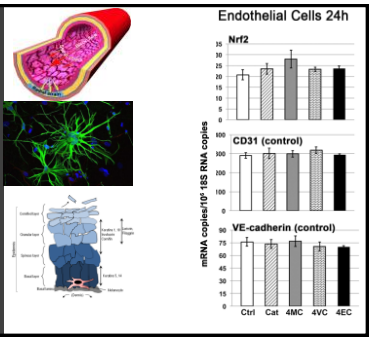
- Microvascular endothelial cells
- 24 hour pre-incubation period prior to H_2O_2 challenge
  - p-value < 0.0001



13

Nrf2 Gene Expression

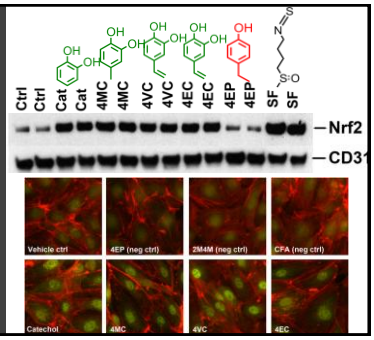
- Modest to no increases in Nrf2 mRNA copies



14

Nrf2 Protein

- Endothelial cells after 24 hour
- Greater Nrf2 nucleus localization in active catechols



15

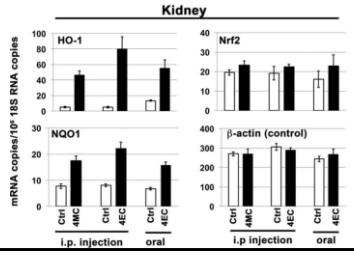
Questions for First Part?

Part 2: *In vivo* Testing and Dietary Bioconversion

Nisha Singh

4MC and 4EC Treatment Induce Nrf2 Target Gene Expression in mice

Both intraperitoneal injection and oral gavage increase target gene expression



1

Lactobacilli: Nature's Bioconverters

- Lactic acid from carbohydrate metabolism
- Fermented foods
- Probiotics

L. plantarum

DIETARY PHENOLIC ACIDS

ENZYME(S)

ALKYL CATECHOLS

*Not all lactobacilli bioconvert to alkyl catechols

2

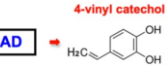
Single Enzyme Bioconversion Reactions

Caffeic acid

PAD

Phenolic acid decarboxylase

4-vinyl catechol



PAD is a microbial enzyme

3,4-Dihydroxy-benzoic acid

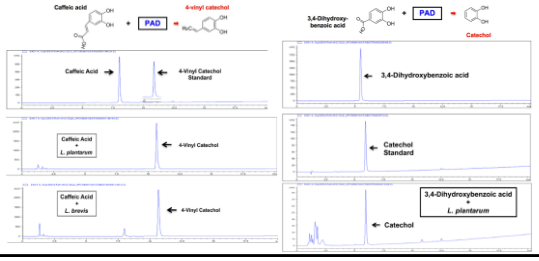
PAD

Phenolic acid decarboxylase

Catechol

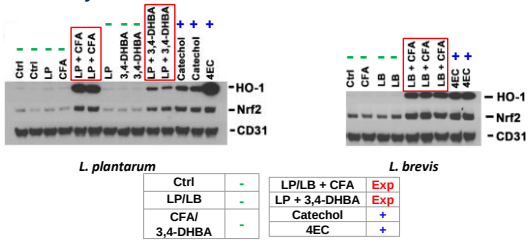
3

Bioconversion by *L. plantarum* and *L. brevis* Analyzed by HPLC



4

Increased HO-1 and Nrf2 Protein Levels in Endothelial Cells after Bioconversion of Dietary Precursors



L. plantarum

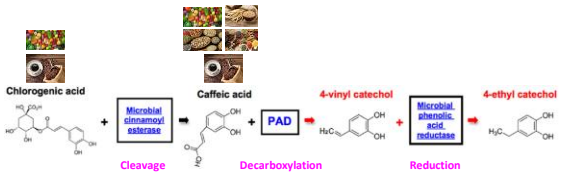
Ctrl	-
LP/LB	-
CFA/ 3,4-DHBA	-

L. brevis

LP/LB + CFA	Exp
LP + 3,4-DHBA	Exp
Catechol	+
4EC	+

5

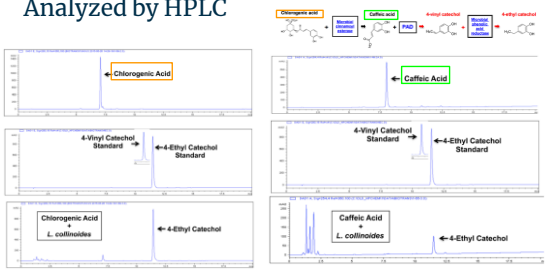
Multi-Enzyme Bioconversion



May be a multispecies undertaking

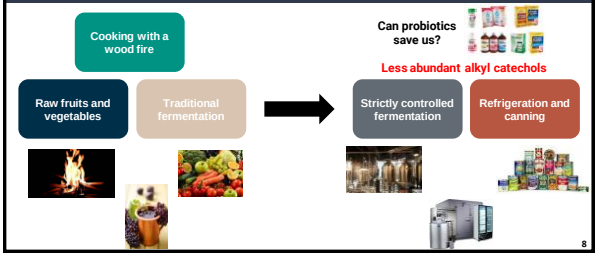
6

Multistep Bioconversion by *L. collinoides* Analyzed by HPLC



7

A Dietary Shift Puts Us More at Risk for Disease



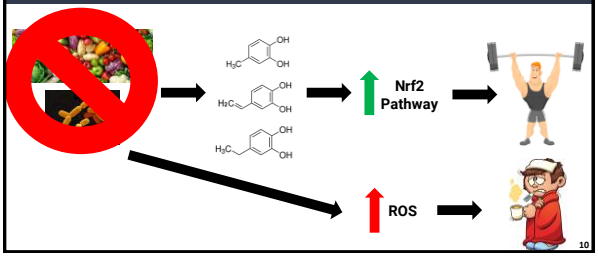
8

Conclusions

- The alkyl catechols are potent activators of the Nrf2 pathway both *in vitro* and *in vivo*
- Microbial bioconversion of inactive alkyl catechol precursors represents a potential important dietary source for these compounds
- Changes in food processing and preservation have eliminated many sources of alkyl catechols from the modern Western diet

9

In Summary



10

Acknowledgements

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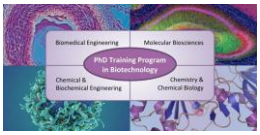
Dr. Martin Yarmush

Evelyn Okeke
Jeffrey Luo
Ryan Guasp
Erika Davidoff



Rutgers Biotechnology Training Grant Fellowship

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Questions?

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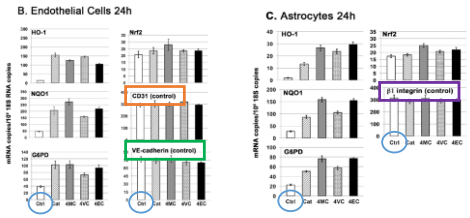
Discussion Topics

Rigor: Looking at controls



What is the purpose of each type of control?
How is each sample also internally controlled?

Other rigor considerations

- How else did they use appropriate controls & negative results to bolster their conclusions?
  - Examples of compounds structurally related to catechols, that do not induce expression of Nrf2 target genes
  - Examples of lactobacilli that do not biotransform caffeic acid to induce expression of Nrf2 target genes
- How many different methods did they use to prove their central point about Nrf2 activation?
  - Answer: at least seven (Figs 2, 6, 7, 8, 10, 12, 16)
- Primary cultured cells vs immortalized cell lines

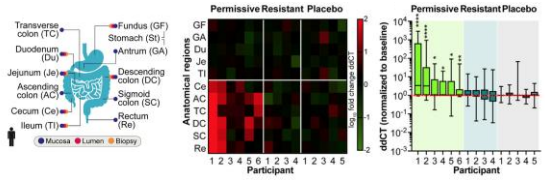
Statistical presentation

How do we feel about the decision to present all statistical information in the captions, and none in the figure itself (e.g. in the form of stars over significant data bar relationships)?

Fig 10. Biotransformation of caffeic acid by *Lactobacillus plantarum* and *Lactobacillus brevis*, as demonstrated with RT-PCR. Y-axis = (mRNA copies)/(10^6 18S rRNA copies). Human dermal microvascular endothelial cells, 24 hours after addition of test samples: Ctrl = control, CFA = caffeic acid, LP = control supernatant from *L. plantarum* incubated with PBS-glucose and filter-sterilized, (LP + CFA) = supernatant from *L. plantarum* incubated with CFA in PBS-glucose and filter-sterilized, LB = control supernatant from *L. brevis* incubated with PBS-glucose and filter-sterilized, (LB + CFA) = supernatant from *L. brevis* incubated with CFA in PBS-glucose and filter-sterilized. CFA and lactobacillus-incubations with CFA were added to a final concentration corresponding to 30 μM CFA starting material (see Methods). 4EC = 4-ethylcatechol positive control (30 μM). Nrf2 target genes = HO-1, NQO1, G6PD. Control mRNAs = CD31 and VE-cadherin. Error bars = ± S.D.; n ≥ 3 for each data point. ***Summary of data analyses and statistical significance:*** As described in previous figures and in the text, induction of the Nrf2 target genes HO-1, NQO1, and G6PD, rather than induction of Nrf2 mRNA, indicates activation of the Nrf2 pathway. Only LP+CFA, LB+CFA, and 4EC (positive control) demonstrated Nrf2 pathway activation by these criteria. For HO-1, NQO1, and G6PD data panels, individual comparisons between Ctrl (or CFA) vs. LP+CFA, Ctrl (or CFA) vs. LB+CFA, and Ctrl (or CFA) vs. 4EC indicated differences that are all extremely significant (p< 0.0001). In contrast, for the HO-1, NQO1, and G6PD panels, individual comparisons between Ctrl vs. CFA, Ctrl vs. LP, and Ctrl vs. LB indicated no significant differences. For Nrf2, Ctrl vs. CFA = small but significant difference (p<0.05), Ctrl vs. LP = not significant, Ctrl vs. LP+CFA = very significant (p<0.01), Ctrl vs. LB = not significant, Ctrl vs. LB+CFA = significant (p<0.05), Ctrl vs. 4EC = significant (p<0.05). Finally, for CD31 and VE-cadherin data sets, statistical analyses indicated no significant differences.

Reproducibility

- Can this study's findings be translated into probiotic product development?



Zmora et al. (2018) "Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features." Cell 174(6):1388-1405.e21.

Ethics

Many researchers have begun to focus on the potential of gut microbiota to ameliorate disease or improve health.

Question 1:

Should the general public try to modify their own biology by taking supplements or probiotics, based on scientific evidence?

Ethics

No probiotics have been approved as live biotherapeutic products. Probiotics are currently treated as supplements (cannot be marketed to cure, mitigate, or prevent disease).

Question 2:

Should the FDA regulate probiotics more stringently, like a food or a drug?

Ethics

Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT

"Of note, dissimilarity from baseline more than tripled during antibiotics treatment in all groups, reflecting the dramatic impact of antibiotics on stool microbiome configuration. [Autologous microbiome transplant] individuals were quickest to return to baseline configuration, with differences in stool composition compared to baseline disappearing as early as 1 day following [autologous microbiome transplant]. In the spontaneous recovery group, significant differences in stool composition compared to baseline abated within 21 days of antibiotics cessation. In contrast, probiotics-consuming individuals did not return to their baseline stool microbiome configuration by the end of the intervention period (day 28), and dysbiosis was maintained even 5 months after probiotics cessation, with all stool samples collected through day 180 remaining significantly different from baseline (two-way ANOVA and Dunnett $p < 0.01$)"

Jotham Suez, Niv Zmora, Gili Zilberman-Schapira, …, Zamir Halpern, Eran Segal, Eran Elinav (2018) Cell 174, 1406–1423

https://doi.org/10.1016/j.cell.2018.08.047

Ethics

The interactions between the organisms of the gut microbiome and their interactions with their host are vastly complex.

Question 3:

Will we ever know enough to safely prescribe or regulate probiotics?

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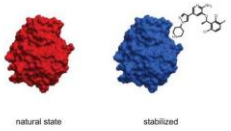
Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay

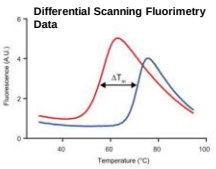
Daniel Martinez Molina,[1*] Rozbeh Jafari,[1*] Marina Ignatushchenko,[1*] Takahiro Seki,[2] E. Andreas Larsson,[3] Chen Dan,[3] Lekshmy Sreekumar,[3] Yihai Cao,[2,4] Pär Nordlund[1,3†]

Zachary Fritz
Topics in Advanced Biotech II
5/3/2019

Background

- Drugs typically bind to target proteins to inhibit or activate them
- Drug developers need to know:
  - Pharmacokinetics (ADME)
    - Drug transport
    - Bioavailibility
  - Pharmacodynamics
    - Potency
    - Affinity
    - Specificity
    - Resistance mechanisms
- Target proteins may be ***stabilized*** (**lower free energy and higher melting point**) by ligands/drugs binding to them

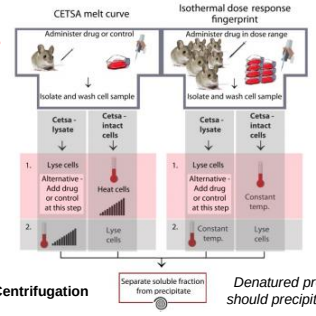




Huber et al. (2015). *Nature Methods*

Cellular Thermal Shift Assay (CETSA)

Dose constant, vary temperature

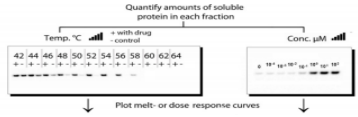


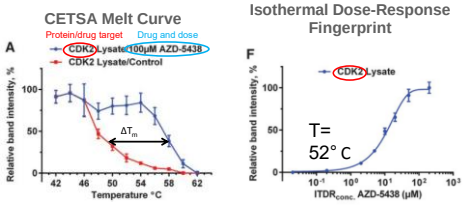
Temperature constant, vary dose

Denatured proteins should precipitate out

CETSA Data

Western Blots

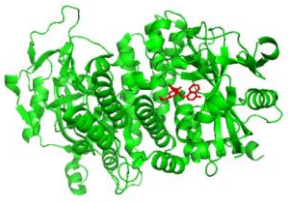




Experimental Outline

1. CETSA in cell lysates
   - Affinity/efficacy
2. CETSA in intact cells
   - Drug transport (intracellular)
   - Drug resistance
   - Specificity
3. CETSA in mice
   - Bioavailibility (intra-tissue)

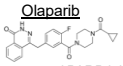
PARP Inhibitors



Thorsell, et al. (2017) *J Med Chem*

- Poly-ADP Ribose Polymerase (PARP): enzyme involved in DNA repair

Olaparib



- FDA-approved PARP inhibitor for some ovarian and breast cancers

Iniparib

- Presumed PARP inhibitor that failed in phase III clinical trials.

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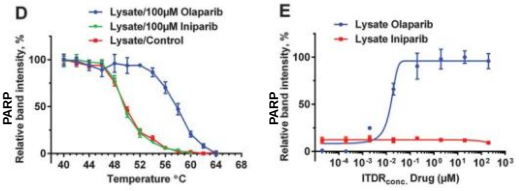
PARP Interaction with Putative Ligands



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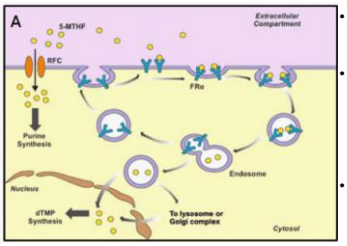
Folate Transport in Cancer



- Cancer cells actively import folates for nucleic acid synthesis
- Reduced Folate Carrier (RFC) and Folate Receptor alpha (FRα) mediate two different forms of active folate transport
- **Folate analog drugs** (Methotrexate, Raltitrexed) can take advantage of these mechanisms

Sanchez del Campo, et al. (2013). *Melanoma*

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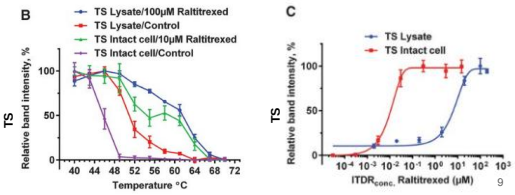
The Effect of Active Transport

CETSA in Cell Lysates vs. Intact Cells

- Thymidylate synthase (TS): involved in DNA synthesis and repair, target of Raltitrexed
- The **intercellular transport** of the drug into the intact cells allowed for a ***lower*** effective concentration compared to the cell lysates (active transport vs. simple diffusion)



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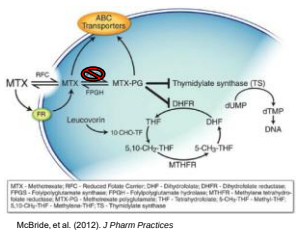
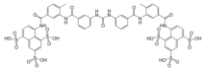
Effect of an Upstream Inhibitor

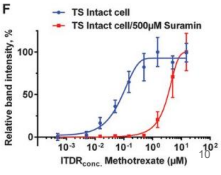


McBride, et al. (2012). *J Pharm Practices*

…thus administration of Suramin requires higher doses of the drug for target engagement



Suramin: inhibits folypolyglutamate synthase (FPGS), which activates Methotrexate…



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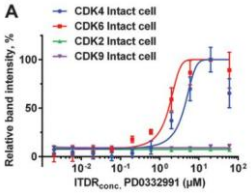
Palbociclib Specificity

IBRANCE® palbociclib 125 mg capsules

IBRANCE® IS THE FIRST FDA-APPROVED MEDICATION in its class. IBRANCE is a targeted therapy known as a CDK 4/6 inhibitor. It is not a traditional chemotherapy. Taken in combination with certain hormonal therapies, IBRANCE works to put the brakes on cell growth in both healthy and cancer cells. This helps slow the progression of cancer, but it can also cause side effects, some of which are serious. Please see Important Safety Information.



- Palbociclib (PD0332991, aka IBRANCE) inhibits cyclin dependent kinases 4 and 6, but not 2 and 9

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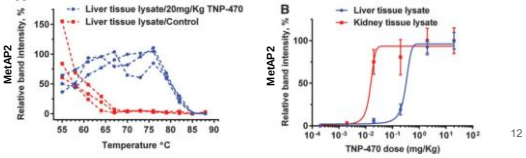
Tissue Bioavailibility

CETSA in Mice

- Mice were injected with TNP-470, an anti-angiogenic inhibitor of methionine aminopeptidase-2 (MetAP2), or control diluent
- After 4 hours mice were sacrificed, liver and kidneys removed
- Lower drug engagement in the liver likely due to presence of catabolic/oxidative enzymes like the cytochrome p450 family



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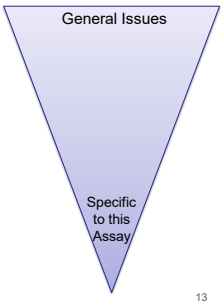
Limitations

- **Thermal shift assays assume drug binding always stabilizes protein**
- Dependent on proteins aggregating and precipitating out of solution upon unfolding
- Drug target/potential targets must be known beforehand
- Western Blots: proteins must be detectable by this method, issue of sensitivity



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Acknowledgements

- Peter Lobel
- Eve Reilly
- Victor Tan
- Larry Cheng
- Matt Tamasi
- The Biotechnology Training Program

NIH National Institute of General Medical Sciences

NIH T32 GM008339

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RESEARCH ARTICLE

Tracking cancer drugs in living cells by thermal profiling of the proteome

Mikhail M. Savitski[1,*,†], Friedrich B. M. Reinhard[1,†], Holger Franken[1], Thilo Werner[1], Maria Fälth Savitski[1], Dirk Eberhard[1], Daniel Martinez Molina[2], Rozbeh Jafari[2], Rebecca Bakszt Dovega[2], Susan Klaeger[3,4], Bernhard Kuster[3,4], Pär Nordlund[2,5], Marcus Bantscheff[1,*], Gerard Drewes[1,*]

[1]Cellzome GmbH, Molecular Discovery Research, GlaxoSmithKline, Meyerhofstrasse 1, Heidelberg, Germany.
[2]Division of Biophysics, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.
[3]Department of Proteomics and Bioanalytics, Technische Universität München, Emil Erlenmeyer Forum 5, Freising, Germany.
[4]German Cancer Consortium, German Cancer Research Center, Heidelberg, Germany.
[5]Centre for Biomedical Structural Biology, Nanyang Technological University, Singapore.

Topics in Advanced Biotechnology II
3rd May 2019
Victor Tan

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Main Objective

Using **mass spectrometry**-based **thermal proteome profiling** to characterize **drug-target interactions**.

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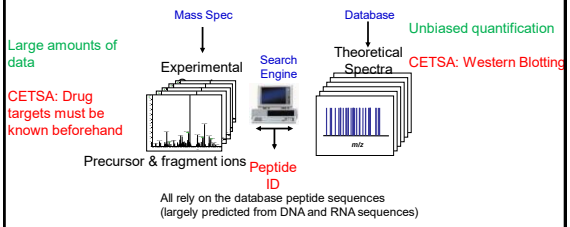
What Mass Spectrometry adds onto CETSA



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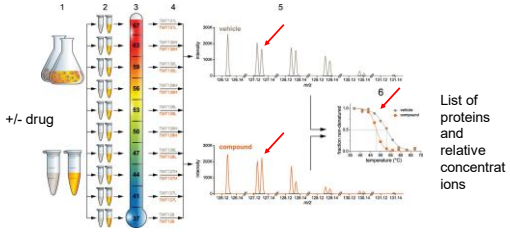
Quantitative thermal proteome profiling under differential conditions



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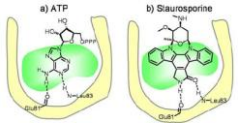
Why Kinase Inhibitors?

Kinases: enzymes that catalyzes the transfer of a phosphate group from ATP to a specified molecule

538 kinases → **37** kinase inhibitors approved, **~150** in clinical trials

- Staurosporine
- GSK3182571
- Dasatinib
- Vemurafenib
- Alectinib



Bhullar KS, Lagarón NO, McGowan EM, et al. Kinase-targeted cancer therapies: progress, challenges and future directions. *Mol Cancer*. 2018;17(1):48. Published 2018 Feb 19. doi:10.1186/s12943-018-0804-2
Meggers, Eric. (2019). **Chemical Biology with Organometallics**.

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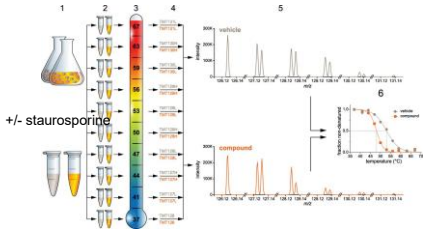
Quantitative thermal proteome profiling under differential conditions



+/- staurosporine

5299 proteins (and relative concentrations) identified

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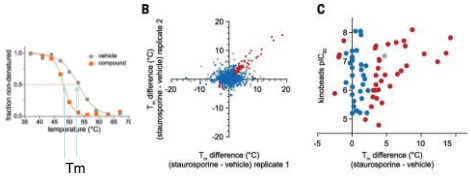
Identified kinases with significant T_m shifts



Kinobeads: beads with immobilized kinase inhibitors, pull down kinases
Obtained list of staurosporine ligands

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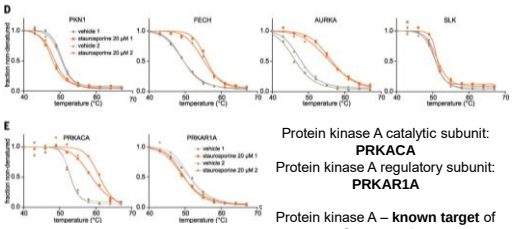
Staurosporine-induced thermal shifts of K562 cellular proteins



Protein kinase A catalytic subunit: **PRKACA**
Protein kinase A regulatory subunit: **PRKAR1A**

Protein kinase A – **known target** of Staurosporine

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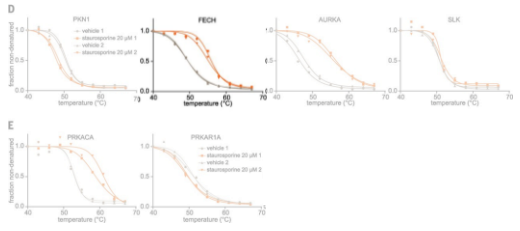
Possible off-target effect on heme biosynthesis pathway?



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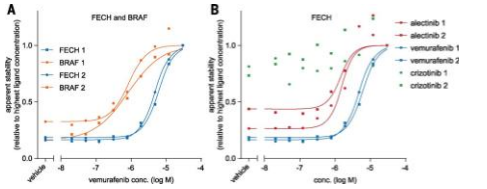
FECH-induced photosensitivity due to vemurafenib and alectinib

Vemurafenib: targets BRAF
Alectinib: targets ALK

Both reported to cause photosensitivity → Photosensitivity linked to FECH deficiency



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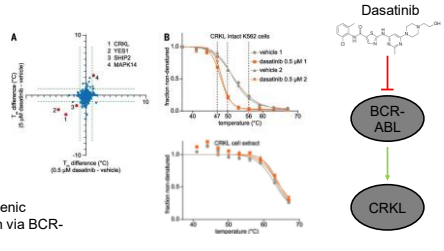
Thermal proteome profiling of dasatinib-treated K562 cells reveal downstream effectors



K562 – oncogenic transformation via BCR-ABL kinase

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Summary

- Cellular thermal shift assay combined with quantitative mass spectrometry identifies drug-ligand interactions by kinase inhibitors.
  - Off-target toxicity
  - Efficacy comparison
- Identification of off-target effects from kinase inhibitors on heme biosynthesis pathway (FECH).
- Identification of downstream effectors affected by BCR-ABL kinase inhibitor dasatinib (CRKL/SHIP2).

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Limitations

- K562 cell line
  - Choose the appropriate model for the appropriate question
  - Systemic drug effects on liver, etc.
- Filtering data
  - "Big data"
  - False positives vs false negatives

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Acknowledgements

- Dr. Peter Lobel
- Zachary Fritz
- Matthew Tamasi
- Larry Cheng
- Eve Reilly

PhD Training Program in Biotechnology

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The National Institute of General Medical Sciences (NIGMS)

NIH T32 GM008339

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Rigor & Reproducibility:

For those that read the paper, how did you feel about the overall thoroughness of these papers? Are there any glaring concerns?

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Rigor & Reproducibility:

Overall we felt as if both papers were thorough. The authors of each paper openly acknowledged the limitations of CETSA as a technique and investigated the reliability of the technique in various environments.

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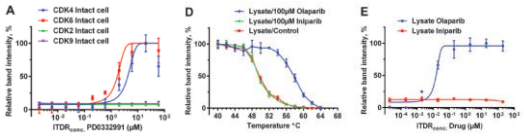

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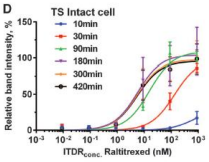

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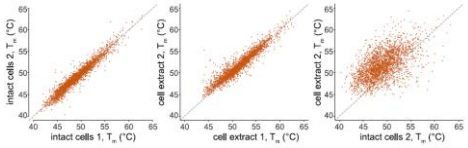

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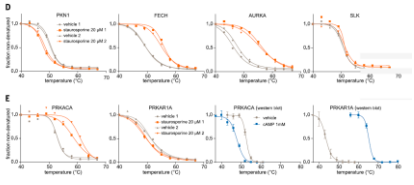

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Rigor & Reproducibility:

While the papers were rigorous, there are important key points to bring up in regards to reproducibility:

1) There are inherent limitations of CETSA as a technique. Not all ligand-protein complexes will increase stability, and not all proteins aggregate.
2) Protein stability is environmentally driven, intact cells and extracts show significantly different stability profiles
3) Big data analysis comes with thresholding and many statistical parameters. Analysis methods play a large role in what is determined as significant and not.

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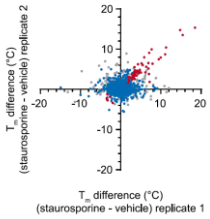

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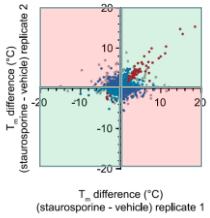

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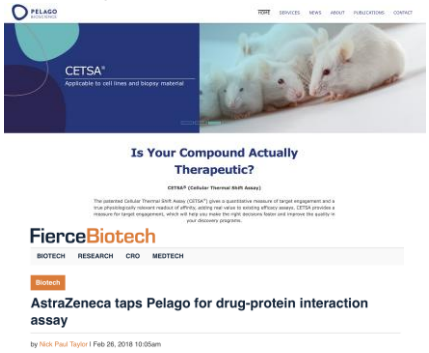
CETSA in Drug Development



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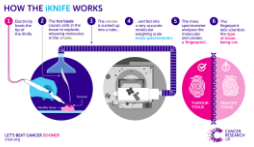
Ethical Considerations of Clinical MS

- Increasing complexity of medical devices and clinical diagnostics. →→ Increase in healthcare costs



- Should state of the art care be available for everyone?
- Or should care be tailored based on the patient's means or socioeconomic status?
- Or should care be determined solely on need?

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848-445-6572

Date	Week	Session Objective (open to schedule changes)
1/23/2019	1	**Introduction:** Familiarize the student with course logistics, technology entrepreneurship and the nature of innovation. • Course overview • Entrepreneurship defined • Emerging technologies • Profile of an entrepreneur • Entrepreneurial types/teams • Entrepreneurial risks • Staged entrepreneurial process
1/30/2019	2	**Analyze the Opportunity:** Innovate and Create the Vision • Observation, problem and need identification • Needs filtering • Ideation and brainstorming • Concept screening • Importance of documentation • Review of venture project technologies and team assignments
2/6/2019	3	**Analyze the Market:** Analyze the Market and Build a Plan, Prepare Industrial Analysis • Innovation types and frameworks • Initial innovation assessment • Market analysis and planning • Market segmentation
2/13/2019	4	**Analyze Competitive Position, Market Forces** • Competitive analysis • Porter's 5 market forces
2/20/2019	5	**Profile the Product/Service** • Profile essentials: 4/7 Ps • Create a brand
2/27/2019	6	**Implement, Scale & Harvest the Venture:** Setting Up the Company, Team Management, Venture Exit • Revenue model development • Business planning outline

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Date	Week	Session Objective (open to schedule changes)
		• Setting up a company • Managing the team • Operational agreements • Exiting the venture
3/6/2019	7	**Communicate the Opportunity:** Build "The Pitch" • Communication guidelines • Pitch elements • Pitch delivery
3/13/2019	8	**Protect the Innovation:** Determine best protection method, File necessary documentation • IP protection overview • Patents defined • Trade secrets defined • Copyrights defined • Trademarks defined • Strategic alliances and licensing agreements G: Kettle, UG: TBD
3/20/2019	SPRING BREAK	
3/27/2019	9	Class Canceled.
4/3/2019	10	**Acquire Financial Resources:** Secure Early Stage and Growth Funding • Valuation overview • Funding sources: equity and non-equity sources • Angel, Venture Capital funding • Funding series **Guest Lecturers:** **David Dalessandro, Consultant** **Sergio Calvo, J&J Innovation, JJDC**
4/10/2019	11	**Material Review**
4/17/2019	12	**Draft Pitch Presentations**

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Date	Week	Session Objective (open to schedule changes)
4/24/2019	13	**Guest Speakers: The Entrepreneurial Experience.** John Crombie: Innovation Strategies from the Inside Out
5/1/2019	14	**Final Pitch Presentations**

BIOENGINEERING IN THE BIOTECHNOLOGY AND PHARMACEUTICAL INDUSTRIES: FUNDAMENTAL AND REAL WORLD PERSPECTIVES

Course Number: 16:125:577 Index Number: 17960

Course Time: Thursday, 5 – 8 PM
BME-128

Description and Objectives:
The goal of this course is to offer students insight into the practical aspects of industrial bioprocessing. Industrial practitioners from various fields of expertise provide lectures and facilitate discussions highlighting problems and issues that engineers and scientists encounter. Topics will vary from year to year but will typically include: drug discovery, drug metabolism, microbial fermentation and mammalian cell culture (optimization and scale-up), monoclonal antibody and vaccine production, gene therapy, downstream purification, formulation, regenerative medicine, stem cell culture, tissue engineering, cellular therapies, regulatory considerations, manufacturing challenges, and clinical research. This course provides students with exposure to topics which are beyond the scope of a purely theoretically-structured course. After taking this course, students should have a better understanding of the challenges that engineers and scientists face in industrial bioprocessing.

Course Directors:

Kristen Labazzo, Ph.D.
Executive Director
Medical Device Development Center
Rutgers University
848-445-6578
kristen.labazzo@rutgers.edu

Martin Yarmush, M.D., Ph.D.
Professor, Department of Biomedical Engineering
Rutgers University
Director, Center for Engineering in Medicine
Massachusetts General Hospital
732-445-4346
yarmush@soe.rutgers.edu

Grading:
25% Class Participation
30% Homework Assignments (submitted on Sakai and due at the beginning of each class)
35% Project (TBD)
10% Short Presentation

Class Preparation: Reading material for each session can be found on the course website:

http://sakai.rutgers.edu
Course: Bioeng Pharma/Biotec 01 Sp18 Assignments

- Course NOT recommended if you expect to miss > 1 class
- You must notify Dr. Labazzo in advance if you will miss a session, and you must submit answers to questions on that session's reading assignment

Topics and Speakers, Spring 2018:

Date	Speaker	Company	Topic
18-Jan	Kristen Labazzo	Rutgers	Class Overview, Science-Driven Business
25-Jan	Brian Murphy	Celgene	"That Can't be Right!" - Real Data in Cell Therapy Development
1-Feb	Greg Russotti	Celgene	Mammalian Cell Culture Scale-Up for Monoclonal Antibody Production
8-Feb	Greg Russotti	Celgene	Live Virus Vaccine Production
15-Feb	George Crotts	GSK	Challenges & Issues in Biopharmaceutical Drug Product Development
22-Feb	Kristen Labazzo	Rutgers	Movie "Extraordinary Measures" and discussion
1-Mar	Kavita Beri	Beri Esthetique	Innovations in Dermaceuticals
8-Mar	Bob Goldberg	CMPI	The Future of Medical Innovation: A History
15-Mar	SPRING BREAK	-----	
22-Mar	Skylar Wolfe	BMS	"Patient Focused Medicine: Developing Innovative Drug Products for Everyone"
29-Mar	Antonio Ubiera	GSK	"Preparative Chromatography for the Purification of Therapeutic Proteins: Process Scale-Up Approaches and Challenges"
5-Apr	Kambiz Shekdar	Research Foundation to Cure AIDS	Discovery and Careers in Biotechnology: A Case Study
12-Apr	Corina White	Amicus	TBD
19-Apr	Mike Daley	OrthogenRX	Development of Cell Based Therapies
26-Apr	Presentations		

Homework:

- Assignments will be given by the speakers selected as an intro to the class topic.
- Answers should be submitted in essay format (1-2 double spaced pages).
- Due at the beginning of class submitted to the Sakai website before 5pm on the day of class.

Presentation:

4-6 powerpoint slides, 5-7 minute presentation including questions summarizing articles or topic proposed by the presenter.

Term Paper:

Due date: **Thursday, 3-May** (Please submit electronic copies by 5pm on that date)

Students are required to submit an abstract and outline on **Wednesday, 9-Mar**. The abstract and outline will be worth 5 points of the total term paper grade. Comments will be returned so both can be updated for the final paper.

Suggested length: 10-15 type-written, double-spaced pages **(***excluding* figures, tables, and references)

Format: Figures and tables are optional but highly desirable; references should be full citations.

The goal of our classroom discussions is to provide you with an introduction to various areas of pharmaceutical bioprocessing. The term paper is a chance for you to learn significantly more about a particular aspect(s) of bioprocess engineering or biotechnology. We encourage you to choose a term paper subject that will encompass more than one of the topics covered during the semester. There are numerous approaches you can take and below are a few examples. We welcome other approaches; let us know your suggestion, and we will let you know if it is acceptable.

Grading Criteria:

	Total Points	Detail
Outline and Abstract	5	Completion on time
Cleary Defined Topic	10	1-3 sentences that clearly defines what will be discussed. Topic should be defined in the abstract along with major conclusions.
Importance of the Topic	10	Appropriate background to demonstrate the broad implications of how the topic impacts our world
Demonstration of Surveyed Literature	25	Key works in the field cited and explained clearly.
Comprehension of Literature	25	Logical opinions formulated about the topic by comparing literature across groups/authors.
Length	5	15-20 pg double spaced- exclude figures and citations
Structure	5	Paper follows logical flow - likely appropriate background, technical review, and formulated assessment
Grammar	5	
Link to Industrial Bioengineering	10	Has anyone considered commercializing? What have been the challenges/results?
Total	100	

Term Paper Topic Examples

1. Choose a recent event or issue, which has received attention in the public domain, and describe the scientific story behind it. This approach would require that you provide sufficient background on the story, as it was told in the public domain, and also delve into the literature to understand the science and engineering behind the story.

2. Write a critical analysis that compares specific technologies used in industrial bioprocessing. Quantitatively evaluate the competing technologies with respect to productivity (yield, purity, etc), scale-up, safety, regulatory factors, and economics.

3. Describe the current challenges of an emerging field in biotechnology and tell us why this field will or will not have a major impact on biotechnology and/or medicine in the future.

Graduate Program and Number: Biomedical Engineering 16:125
Course Number and Title: 16:125:578
Interdisciplinary Biostatistics Research Training For Molecular And Cellular Sciences: Enhancing Rigor And Reproducibility"

Week	Topic	Reading
1	**Overview and Descriptive Statistics.** Type of data, graphic presentation, central tendency and dispersion, introduction to R, introduction to GraphPad Prism.	1 Ch. 1,2
2	**The Peanut Lab I.** Students will generate data of peanut length and weight to demonstrate variability within and between groups. They will use GraphPad Prism to present their data.	
3	**Probability and Distributions.** Probability, conditional probability, binomial and normal distribution.	1 Ch. 3,4
4	**Estimation.** Sampling distribution, confidence interval (population means and proportions), sample size estimation based on proportions.	1 Ch. 5,6
5	**Hypothesis Testing**. Type I and type II error, steps of performing hypothesis testing (hypothesis testing on population means, hypothesis testing on population proportions, *z* and *t*-statistics), power and sample size estimation.	1 Ch. 7
6	**The Shell Lab.** Students will be asked to measure the length and width of cold and warm water seashells and determine if there is statistical difference between the two groups.	
7	**Analysis of Variance.** Comparisons between and among means, multiple comparisons.	1 Ch. 8
8	**Correlation and Regression.** Correlation and simple linear regression.	1 Ch. 9
9	**Multiple Linear Regression and Logistic Regression.** Multiple linear regression, model building and diagnosis, logistic regression.	1 Ch. 10,11
10	**Analyze my Data Lab.** Students will analyze data that they have generated using R and GraphPad Prism to analyze and graphically display the results.	
11	**Nonparametric Statistics.** Sign test, Wilcoxon sign rank test, Wilcoxon rank sum test, Kruskal-Wallis test.	1 Ch. 13
12	**Survival Analysis**. Kaplan-Meier procedure, Log-rank test, Cox proportional hazard model.	1 Ch. 12
13	**Biostatistics in the Genomic Age.** Microarray data analysis.	
14	**Reading the Scientific Literature.** Use of statistical analysis in the scientific literature, misuse of statistical analysis in the scientific literature.	Handouts
15	**Student Presentations.** Students will present preliminary statistical design and data analysis plan for their thesis projects.	

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School of Engineering
Professional Preparedness in Biotechnology
16:125:579:01
(3 credit)
BME 126
Updated 5/31/2019

Course Background/Overview
Although current courses in the typical graduate curriculum appropriately deliver strategic discipline-based learning for life science and engineering graduate students, the broader biotech and health science industry further demands that scientists be prepared to serve a variety of distinct functions within the life and biomedical sciences ecosystem, and to understand broader developmental aspects of the business of science and engineering in a professional environment. Many scientific professionals, while experts in their respective fields, have little academic/professional background in business management; skills that ensure that scientific projects and research are implementable, feasible and sustainable. In addition, these skills work to expand scientists' and researchers' professional reach and help them to realize their true career potential. This course entitled, "Professional Preparedness in Biotechnology" will enhance students' competitive skills and introduce additional layers of specialized competence enabling immediate contribution within diverse organizations in the life and biomedical sciences commercial sector. Students will develop business, communication, management, (and other), and skills.

This course will be offered during the first 2019 Summer Session (May 28, 2019 through July 2, 2019), over a 6-week period, on Tuesdays and Thursdays for sessions of 4 hours each with direct student contact. Each session will be comprised of lecture, followed by lab in which students will have hands-on experience with the concepts introduced, as they review and analyze case studies specific to various professional environments and challenges. They will then present recommendations to the class to seed group discussions and further role-play.

Textbooks: *Various, (licensed) copies of relevant sections scanned for student use.*

Instructional Methods: This course will utilize various learning modes to ensure that students are engaged and successfully integrate the concepts learned with their professional area(s) of focus. Course concepts will be emphasized in (at least) three different ways:

1. The lecture portion of the course introduces appropriate concepts in an interactive, engaging format. Basic tenets are presented and students are asked to apply the concepts to their personal experiences and to highlight the differences in application in academic and corporate environments.
2. During the lab portion, students are introduced to a case study profiling genuine situations in which the key concepts learned are prominently cast. The case studies, gleaned from current business situations and made anonymous to minimize bias, describe business situations that require decisions and/or action plans relative to topics such as accounting, marketing, finance, quality control, operations research, research and development (in an industry setting), project management and others. Students are grouped into teams and each is assigned one of the "characters" introduced in the case study. Through group discussion and role-play, allowing team members to consider multiple solutions, the team crafts a strategic action plan. During this lab portion, the instructor facilitates, rather than directs, student interaction and mastery of the concepts highlighted and students are required to present their results during the remaining 30 minutes of the class.
3. A final project will require that students work individually to present a case study focused within their area of professional interest and analyze the actions and inactions relative to the concepts taught in class.

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4. In addition to work within formal class time, online forums/blogs, will be available as a venue for students to participate in discussions with class instructors, share their opinions and experiences, and debate with professors and fellow students. The forums will be open for 7 days, with (one of the) professors opening the discussion with a topic or question about which participants will share their thoughts and answers, providing the students opportunity for reflection, personal connection with the material and understanding of how the concepts learned can be applied to other, varied circumstances. Available 24 hours a day, the format will allow each student to make his/her discussion contributions at a time of his/her convenience.

Using this multi-pronged approach, students will learn to think outside the "researcher's box" and understand how business functions; an indispensable skill for all scientists that wish to navigate the boundaries between research and industry.

Course Assignments and Grading: Student performance will be evaluated through participation class discussions, in group projects, presentations of their case study analyses and participation in forums/blogs.

Instructors: As the course is comprised of sessions focused upon unique individual aspects of professional preparedness, several instructors will be involved in its delivery. To address certain business or legal-intensive topics, senior faculty member instructors will be recruited from the Rutgers Business School, Rutgers Law School, and others to ensure that the required expertise is represented. Where no expertise is resident within the Rutgers schools, outside instructors (with affiliation/history with the Rutgers), will be recruited to teach specific sessions.

Course Objectives

1. Promote an understanding of key technology professional skills and effectiveness of delivery for same
2. Equip students with skills and knowledge for post-graduation professional placements
3. Improve qualifications of students in preparation for employment within the field of biotechnology

Course Outline

The course provides the students with the following knowledge and perspective:

- **Course Overview:** curriculum review, case-based analyses to identify professional success factors
- **Life and Biomedical Sciences Ecosystem:** overview of the life and biomedical sciences marketplace, review of industry strategies, analysis of the economic environment
- **Managing Communications:** individual skills assessment, team dynamics, decision-making, responsibility, interpersonal skills, presentation/pitching, communication vehicles
- **Project Planning and Management:** phased development process, quality gates, project management
- **Customer Focus:** customer requirements, profiles of economic stakeholders, decision-makers, payers, marketing, sales
- **Financial Management:** cash flow, investment evaluation, risk & return, financial statements
- **Operations Management:** business logistics, demand planning, global sourcing
- **Risk Management, Quality and Safety:** risk analysis**,** good manufacturing practices (GMP), good lab practices (GLB), quality control, quality assurance
- **Regulatory Processes:** market clearance pathways for drugs, biologics, medical devices and combination products (laws, regulations, and regulatory agencies)
- **Organizations and Partnerships:** internal and external partnerships, negotiating corporate silos
- **Ethics in Biotechnology:** TBD
- **Student Presentations.** Students will submit, and present, case study analyses relevant to their current area of professional interest and highlighting lessons learned throughout the course.

Center for Innovative Ventures of Emerging Technologies

School of Engineering
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Note that these topics are not assigned to specific classes as some may require more than one session to complete; therefore, the exact sequence remains fluid. Within coverage of these topics areas, we build upon the following critical skills for professional success:

- Strategic thinking
- Art of selling, persuasion and motivation
- Oral and written communications

The course consists of lecture with extensive participation between students and the instructor. Concepts are inter-mingled with practical applications whereby students are challenged to apply an academic concept to real-world professional context.

Assignments
Students will be assigned to teams to work on case analyses provided by the instructor, with review of articles and role-play scattered throughout the individual classes. Throughout the course, readings are assigned and relevant discussions held during subsequent classes. For some of these reading assignments, written responses to questions will be required.

Prerequisites
Graduate student populations who seek to learn skills recommended for success within the biotechnology professional organizations.

Textbook(s)
There are no textbooks required for this course. Readings are provided throughout the sessions.

Grading Criteria

• Overall Class Attendance, Contribution and Discussion	25%
• Session-Focused Case Study/Participation	20%
• Contribution to Blog	15%
• Final Case Study/Presentation	25%
• Final Essay	15%

Note that class attendance is mandatory. Each student is allowed one unexcused absence and, in the event that he/she is absent two or more times, he/she will forfeit 10% of the grade allocated for class contribution/participation (equal to one letter grade). Students are expected to come to class having read the assigned material, completed the assignment, and well prepared to engage in dialogue regarding the assigned material. All reading and other preparatory assignments must be completed by their due date(s).

TOPICS IN BME: APPLICATIONS IN MEDICAL DEVICE DEVELOPMENT

Course Number: 16:125:575 **Index Number: 21374**

Course Time: Thursday, 5 – 8 PM **Room**: BME-116

Description and Objectives:

This course will provide students insight into the practical aspects of medical device applications, and introduce business concepts as they relate to medical devices from a realistic industrial perspective. Representative fields including but not limited to cardiovascular, orthopedics, diagnostics, imaging, rehabilitation, and dental will be covered. Within each field, topics such as market and design considerations, FDA pathway, clinical trial requirements, manufacturing/QA/QC, and post-market considerations will be touched on. Industrial practitioners provide lectures and facilitate discussions highlighting problems such as manufacturing issues or project management challenges that engineers and scientists may encounter when dealing with the medical device industry.
After taking this course, students should have a better understanding of the challenges that engineers and scientists face in the medical device industry and gain an appreciation for the practical applications of their academic studies.

Course Director:

Kristen Labazzo, Ph.D., MBA
Executive Director, Medical Device Development Center
Asst. Professor of Practice
Rutgers University
848-445-6578
kristen.labazzo@rutgers.edu

Major Textbooks and Other Reference Materials:

Relevant reading material will be provided by each lecturer.

Criteria for student grading:

Component	Percent
Class Participation	25%
Homework	30%
New Device Executive Summary	35%
Presentation	10%
Total	**100%**

Homework:

- Questions will be posed to by the speakers related to materials selected as an intro to the class topic.
- Answers should be submitted in essay format.
- Due at the beginning of class to ensure preparedness for the lecture and active participation.

Class Preparation: Reading material for each session can be found on the course website:

https://sakai.rutgers.edu/portal/site/80ba05a5-6afa-48ed-8375-f80ee8676911

- Course NOT recommended if you expect to miss > 1 class
- **You must notify** Dr. Labazzo in advance if you will miss a session, and you must submit answers to questions on that session's reading assignment

Topics and Speakers, Spring 2019:

24-Jan	A) Introduction to Course Objectives B) "The Bleeding Edge" Documentary and Discussion	*Kristen Labazzo*	Rutgers
31-Jan	Medical Devices: An Overview of Generally Used Standards & Guidances	*Rosemarie Logan*	Rlogan Consulting (Regulatory Science)
7-Feb	Historical FDA Perspective for Medical Device Development	*Jordan Katz*	Orthobond
14-Feb	Dental Applications for Medical Devices	*Josh Simon*	Spiral Medical Development
21-Feb	Orthopedic Reconstruction Devices and Bone Void Fillers	*Paul Viola*	Quantum Concepts
28-Feb	Wheelchairs and seating: Promoting abilities through understanding and innovation	*John Reck*	Matheny Medical and Educational Center
7-Mar	Process for Scouting and Evaluating New Technologies for Medical Diagnostics	*Lance Ladic*	Siemens
14-Mar	Product Management Across Life of a Medical Device from Innovation to Life Cycle Management	*Nasir Uddin*	BD
21-Mar	SPRING BREAK-NO CLASS		
28-Mar	Role of Medical Devices in Healthcare Associated Infections	*David Dalessandro*	Johnson & Johnson, retired
4-Apr	Cardiovascular Medical Devices	*Natalie Macon*	Allergan
11-Apr	The Integration of Design and Manufacturing for Medical Devices	*Meg Smith*	Stryker
18-Apr	Wound Closure Products	*Carlos Caicedo*	Orthobond
25-Apr	Panel Discussion (physician, patient, clinical, researcher, engineer, etc all at one table!)	variety TBD	variety
2-May	Medical Device Presentations	Guest judges	

Medical Device Proposal:

Students will form groups and have the opportunity to propose a novel medical device. The idea does not have to be realistic, so long as it can be appropriately justified and a convincing argument can be made. Students are to prepare an executive summary for their medical device which should include the following elements:

- Opportunity: what is the unmet need that your device fulfills?

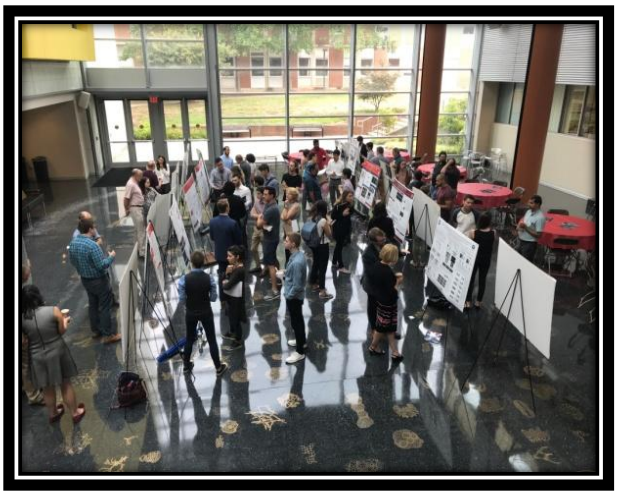
- Value Proposition: how will your device be better? What value does it bring to the community you are serving?
- Market Size: who are your customers and how large is the population? If there are comparable products, how many are sold a year?
- Development: What are your big design hurdles? User Needs, Design Inputs, Performance Requirements??
- Investment Opportunity: how much money are you looking to generate? Can the product be reimbursed through health insurance to make it more attractable to physicians?
- Competition and Barriers to Entry: what are some competitive products? Are there other barriers such as FDA issues or clinical trial difficulties?
- Exit Strategy:
- Freedom to Operate: are there patents which may prevent you from making this product?
- Regulatory: what FDA classification will your device have? What will your clinical trials look like? Enrollment size?

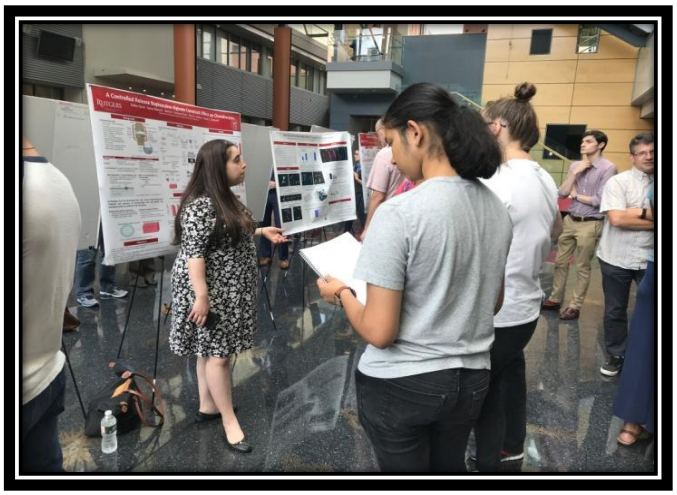
The executive summary should be no more than 2 pages.

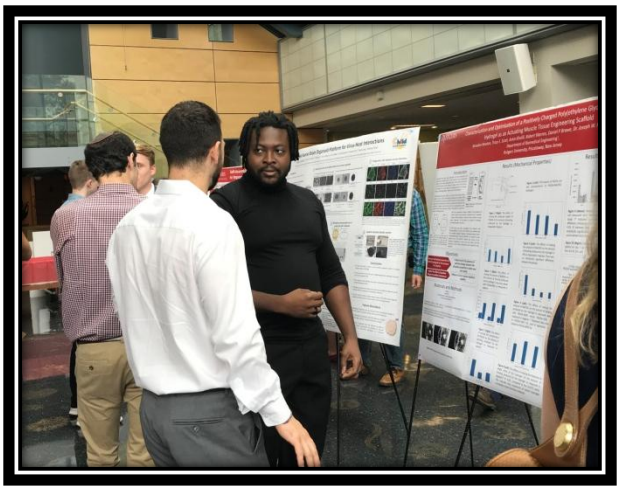
Presentation:
Groups will have the opportunity to present their medical device concept to the class. Each presentation should contain 7-10 powerpoint slides. Presentations will be graded on relevance, novelty, justification of idea, attention to detail, and how well the presentation is organized and delivered. Previous industrial speakers will be invited back to serve as guest judges.

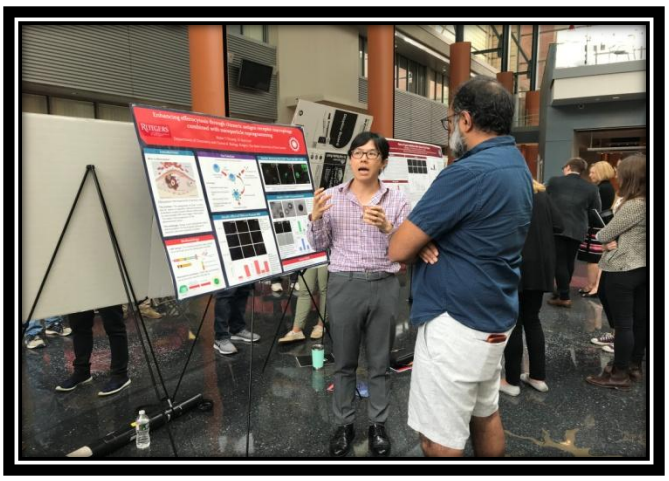
Academic Integrity:
Students are expected to familiarize themselves with and adhere to the University policy on academic integrity at: http://academicintegrity.rutgers.edu/academic-integrity-policy/

ANNUAL SYMPOSIUM









AGENDA

Continental Breakfast…… 9:30 am

Introduction……10:00 am

Dr. Martin Yarmush
Co-Director, Biotechnology Training Program
Rutgers University

Keynote Address……10:30 am

Dr. Naomi Murray
Director, Advanced Operations for Additive Technology
Stryker Corporation

Poster Session…… 11:30 am

Lunch…… 12:30 pm

Poster Awards and Closing Remarks…… 1:30 pm

KEYNOTE SPEAKER

Dr. Naomi Murray

Director
Advanced Operations for Additive Technology
Stryker Corporation

Naomi Murray, Ph.D., Director, Advanced Operations for Additive Technology, began her career with Stryker Corporation developing new technology for use in Orthopaedic applications and worked to develop, characterize and validate new technology such as Tritanium®, Stryker's Additive Manufacturing Technology, and other advanced manufacturing technologies. For over 15 years, Dr. Murray has worked in the medical technology industry in areas spanning technology development from early phase R&D, global regulatory approval and device manufacture. Dr. Murray earned her Sc.B. in Materials Science and Engineering from Brown University and her Ph.D. in Materials Science and Engineering from Northwestern University

STUDENT POSTERS

Jeremy Anderson
Biomedical Engineering
Dr. Li Cai

Andrew Boreland
Neuroscience
Drs. Zhiping Pang and Peng Jiang

Alexandra Burr
Biomedical Engineering
Dr. Biju Parakkadan

Larry Cheng
Cellular and Molecular Pharmacology
Dr. Justin M. Drake

Skylar Chuang
Chemistry and Chemical Biology
Dr. Ki-Bum Lee

Mollie Davis
Biomedical Engineering
Dr. Martin Yarmush

Emily DiMartini
Biomedical Engineering
Dr. David Shreiber

Zachary Fritz
Biomedical Engineering
Dr. Martin Yarmush

Josh Leipheimer
Biomedical Engineering
Dr. Martin Yarmush

Jeffrey Luo
Chemistry and Chemical Biology
Dr. KiBum Lee

Ileana Marrero-Berríos
Biomedical Engineering
Dr. Martin Yarmush

Ilija Melentijevic
Molecular Biology and Biochemistry
Dr. Monica Driscoll

Yoliem Miranda Alarcón
Biomedical Engineering
Dr. David Shreiber

Jenna Newman
Biochemistry and Molecular Biology
Dr. Andrew Zloza

Brandon Newton
Molecular Biology and Biochemistry
Dr. Joseph Freeman

Anton Omelchenko
Cell Biology & Neuroscience
Dr. Bonnie L. Firestein

Misaal Patel
Biomedical Engineering
Dr. Li Cai

Xiomara I. Perez
Biomedical Engineering
Dr. Martin Yarmush

Christopher Rathnam
Chemistry and Chemical Biology
Dr. Ki-Bum Lee

Eve Reilly
Molecular Biology and Biochemistry
Dr. Mikel Zaratiegui

Nisha Singh
Neuroscience
Dr. Bonnie Firestein

Victor M. Tan
Pharmacy
Dr. Justin M. Drake

Rahul Upadhya
Biomedical Engineering
Dr. Adam Gormley

Caroline Wood
Biomedical Engineering
Dr. Jay Sy

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Name/Department	Current or Last Known Position
Patricia Darcy, Biochemical Engineering	Associate Professor, Chemical Engineering, Lafayette College
Frank Goveia, Microbiology	Head of Sales, Trio Health, CA
Michael Sacco, Pharmaceutical Sciences	Vice President of Quality for Medical Affairs, Regulatory and Safety, Novo Nordisk
Jean Boyer, Biochemical Engineering	Senior Director Analytical Sciences, Inovio Pharmaceuticals Inc.
Vaughn Cleghon, Microbiology	Associate Professor, Department of Pediatrics, University of Cincinnati
Ramona Lloyd, Microbiology	President and Principal Consultant, CymReg Consulting, LLC
Maria Lee, Pharmaceutical Sciences	Research Scientist, Advanced Care Products, Ortho Pharma
Diane Zimmerman, Computer Science	Technical Writer and Editor, Self-employed, CO
Carlos Aparicio, Biochemical Engineering	CEO and President, ImmunoSite Technologies, FL
Nathan Busch, Biochemical Engineering	Attorney-at-Law, Anovus LLC, MN
Amlan Dutta, Biochemical Engineering	Vice President, Biologics Quality, Celgene, NJ
Susan Harlocker, Molecular Biology & Biophysics	Senior Patent Agent, Second Genome Inc., CA
Deena Oren, Chemistry	Manager, Structural Biology Resource Center, The Rockefeller University
Maura Collins Pavao, Microbiology	Professor, Biology, Worcester State University
Mark Riley, Biochemical Engineering	Associate Dean for Research, University of Nebraska
Connie Schall, Biochemical Engineering	Professor & Graduate Director, Chemical & Environmental Engineering, University of Toledo
Nancy Sladicka (Iler), Molecular Genetics	President, US Operations, Nucleus Global , NY
Srikanth Sundaram, Biochemical Engineering	President and Chief Scientific Officer, MAIA Pharmaceuticals , NJ
William Thorpe, Biochemical Engineering	Area Director, Club Z! In-Home Tutoring Services, Winchester, MA
Ashish Upadhyay, Biochemical Engineering	Senior Research Biochemical Engineer, Merck & Co, PA
Kenneth Valenzano, Pharmacology	Vice President Pharmacology and Biology, Amicus Therapeutics, NJ
Madhaven Vasudevan, Biochemical Engineering	Vice President, Analytics Solutions, GENPACT, CA
David Odde, Biochemical Engineering	Professor, Biomedical Engineering, University of Minnesota
Paul Olson, Molecular Genetics	President and Co-founder, Kypha Pharma, Inc.
David Powers, Biochemistry	Senior Principal Research Scientist, Abbott BioTherapeutics, CA
Maria Ortiz Rivera, Microbiology	Scientific Support Call Center Leader, GE Healthcare, MA

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Amit Roy, Biochemical Engineering	Group Director, Clinical Pharmacology & Pharmacometrics, Bristol-Myers Squibb, NJ
Myrna Uytingco, Biochemical Engineering	Physician (Medicine), Lakeside Community Healthcare, Providence Holy Cross Med Ctr, CA
Clelia Biamonti, Biochemistry	Executive Director, Overlook Foundation, NJ
David Lamberto, Biochemical Engineering	Associate Director, Engineering, Merck & Co, NJ
Elizabeth Powell, Biochemical Engineering	Program Director, National Institute on Alcohol Abuse and Alcoholism, MD
Greg Russotti, Biochemical Engineering	Vice President, Cell Therapy Technical Operations, Celgene Cellular Therapeutics, NJ
Bruce Weaver, Biochemical Engineering	Process Development Leader, Adello Biologics, NJ
Shiun-Kwei Chiou, Molecular Biology	Adjunct Professor, National University, Principal Scientist, Department of Veteran Affairs, CA
Joseph Le Doux, Biochemical Engineering	Associate Professor & Associate Chair, Biomedical Engineering, Georgia Tech
Colette Ranucci, Biochemical Engineering	Executive Director, Biologics External Mfg.,Merck & Co, PA
Hsin Chien Tai, Biochemical Engineering	Technical Product Manager/Senior Material Designer, Monlnlycke Health Care, NJ
Charlie Chang, Biochemical Engineering	Senior Investment Officer, Missouri State Employees' Retirement System
Hany Michail, Biomedical Engineering	Physician (Ophthalmology), The University of Texas Southwestern Medical Center
Matthew Pellegrini, Biochemistry	Manager, Drug Discovery, PTC Therapeutics, NJ (Deceased 2006)
Seshu Pedapudi Tyagarajan, Biochemical Eng	Director, Cell and Gene Therapies, Novartis Pharmaceuticals, NY
Petra Archibald, Biochemical Engineering	Facilitator & Mediator, Soliya/Institute for Mediation & Conflict Resolution, NY
Lori Herz, Biochemical Engineering	Professor of Practice, Bioengineering, Lehigh University, Owner Hez Biotechnology Consulting LLC, NJ
Todd Muccilli, Biochemical Engineering	Director of Operations, Integrated Process, Merck & Co, PA
Jane Tjia (Atkins), Biochemical Engineering	Senior Director, Portfolio & Program Management, Wve Life Sciences, MA
Albert Alexander, Biochemical Engineering	Senior Scientist, AstraZeneca, PA
Aquanette Burt, Biochemical Engineering	Senior Manager, R&D Nima Labs, CA
Elizabeth Shen, Biochemical Engineering	Technical Sales Manager, Chemische Fabrik Budenheim, DE
Deanna Thompson, Biochemical Engineering	Associate Professor, Biomedical Engineering, Rensselaer Polytechnic Institute
C. Alves, Molecular Genetics & Microbiology	Medical Student, Univ of Texas
Leonard Edelstein, Mol Genetics & Microbiology	Research Assistant Professor, Cardeza Foundation for Hematologic Research, Thomas Jefferson University
Joseph Freeman, Biomedical Engineering	Associate Professor, Biomedical Engineering, Rutgers University

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Scott Banta, Biochemical Engineering	Professor, Chemical Engineering, Columbia University
Eric Hacherl, Biochemical Engineering	General Manager, Noramco, DE
James McCarthy, Mol Genetics & Microbiology	Staff Scientist, J. Craig Venter Institute, Scripps Institute of Oceanography, CA
Mary Lynn Mercado, Pharmacology	US Group Head Regulatory Medical Writing, Novartis Pharmaceuticals Corporation, NJ
Annmarie Pacchia, Microbiology	Vice President, Research and Project Administration, Memorial Sloan Kettering Cancer Center, New York, NY
Thomas Brieva, Biochemical Engineering	Senior Director, Cell Therapy Process Development, Celgene Cellular Therapeutics, NJ
Paul Gong, Chemistry	Senior Professional Staff, John Hopkins University Applied Physics Laboratory
Sean Hanlon, Molecular Genetics & Microbiology	Associate Director, Center for Strategic Scientific Initiatives, National Cancer Institute
Elizabeth Manheim, Genetics	Lecturer, Biology, Kean University
Susan Maskery, Biochemical Engineering	Biotechnology Professional, MN
Kristine Schmalenberg, Chemistry	Director Clinical for Clinique, Estee Lauder, NY
Jintae Lee, Biochemical Engineering	Assistant Professor, Chemical Engineering, Yeungnam University, Korea
E.J. Amato-Pavlik, Biochemistry	Senior Project Manager, Amgen, Inc, CA
Michael Baran, Biochemistry	Senior Director, Business Operations & Scientific Affairs, Pfizer, NY
Michele Burley, Molecular Genetics & Microbiology	Research Scientist, Germ Guard Lighting, NJ
Brian Geldziler, Molecular Genetics & Microbiology	Director, Medical Writing, Otsuka Pharmaceutical Companies, NY
Paloma Pimenta, Biochemical Engineering	Senior Technical Associate, R&D Personal Care Product Development, Colgate Palmolive, NJ
Andrew Roberts, Mol Genetics & Microbiology	Director, CERA and CSAFF, ILSI Research Foundation, VA
H. Chen, Chemistry	Postdoctoral Fellow, Massachusetts Institute of Technology
Justin Lacombe, Biochemical Engineering	Director, Pharmaceutical Development, Experic Pharma, PA
Eric Semler, Biochemical Engineering	Director, R&D, Musculoskeletal Transplant Foundation, NJ
Ram Sharma, Biochemical Engineering	Research Engineering and Scientist, BioRestorative Therapies Inc., NY
David Snyder, Biochemistry	Professor, Chemistry, William Paterson University, NJ
Joseph Vitolo, Biochemical Engineering	Deceased 2006
Carlos Caicedo, Biomedical Engineering	Director R&D, Orthobond, NJ
Tim Maguire, Biomedical Engineering	Director Corporate Development, LUYE Pharma Group, MA

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Jason Maikos, Biomedical Engineering	Director, Gait and Motion Analysis Laboratory Manhattan VA Medical Center
Erik Novik, Biomedical Engineering	Director, Business Development, Crown Bioscience, Inc., NJ
Sam Phillips, Biomedical Engineering	Health Scientist, Center of Innovation on Disability & Rehabilitation Research, Veteran Affairs, VA
James Voordeckers, Mol Genetics & Microbiology	Research Associate, University of Oklahoma
Eddie Davis, Biochemical Engineering	Engineer, Epic Systems Corp, WI
Chris Gaughan, Biochemical Engineering	Scientist, CLG, LLC, CA
Frances Gratacos, Mol Genetics & Microbiology	Coordinator International Education, Centro Boliviano Americano, NY
Keirnan Lamarche, Biochemical Engineering	Senior Researcher, Bristol Myers Squibb, NJ
Natesh Parashurama, Biochemical Engineering	Assistant Professor, Chemical and Biological Engineering, SUNY Buffalo
Nina Rodriguez-Pinto, Biochemical Engineering	Investigator, Sanofi, NJ
Alan Sasso, Biochemical Engineering	Statistician, US Environmental Protection Agency, VA
Harini Sundararaghavan, Biomedical Engineering	Assistant Professor, Biomedical Engineering, Wayne State University
Eric Yang, Biomedical Engineering	Senior Investigator, Novartis Institutes for BioMedical Research, PA
Leilani Del Rosario, Chemistry	Senior Research Chemist, Church and Dwight, NJ
Maria Della-Valle, Biochemistry	Associate Principal, Amicus Therapeutics, NJ
Stephen Guzikowski, Biochemical Engineering	Research Scientist, Bristol-Myers Squibb, NJ
Nicole Iverson, Biomedical Engineering	Assistant Professor, Biological Systems Engineering, University of Nebraska
Dominick Naczynski, Biomedical Engineering	Senior Vice President, Boxer Capital LLC, CA
Ronald Perez, Pharmacology	Research Analyst, Terumo Medical Corporation, NJ
Eric Wallenstein, Biomedical Engineering	Associate Director, Merck & Co., NJ
Chris Langhammer, Biomedical Engineering	Resident Physician, Orthopaedic Surgery, UCSF
Norman Lapin, Biomedical Engineering	Research Associate, Baylor College of Medicine, TX
Joseph Moloughney, Pharmacology	Scientist, Merck & Co., NJ
Jean-Pierre Dolle, Biomedical Engineering	Postdoctoral Fellow, Neuroscience, University of Pennsylvania
Kevin Nikiticzuk, Biomedical Enginnering	Principal at Heidrick Consulting, NY
Melinda Kutzing, Biomedical Engineering	Managing Partner, Bionest Partners, New York
Eric Sweet, Neuroscience	Assistant Professor, Biology, Westchester Univ of Pennsylvania

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Jeff Barminko, Biomedical Engineering	Associate Scientist, Ichan School of Medicine at Mount Sinai, NY
Nripen Sharma, Biomedical Engineering	Scientific Writer, Epigeneres Biotech, India
Jonathan Gonzalez-Flores, Molecular Genetics	Postdoctoral Fellow, Molecular Oncology, Ponce School of Medicine, Puerto Rico
Shirley Masand, Biomedical Engineering	Management Consulting, Accenture, NY
Lawrence Sasso, Biomedical Engineering	Lead Engineer and Chief Technical Officer, Genesis Technologies, TX
Jillian Whidby, Chemistry	Medical Writer, RRD International, LLC, PA
Serom Lee, Biomedical Engineering	Consultant, Cello Health BioConsulting, NJ
Daniel Lewis, Biomedical Engineering	Associate Consultant, Cello Health BioConsulting, NJ
Frank Macabenta, Pathology	Postdoctoral Research Scholar, Biology, California Institute of Technology
Adriana Martin, Pharmacology	Medical Resident, Universtiy of Arizona
Nir Nativ, Biomedical Engineering	Medical Device R&D Leader, Ethicon, Johnson & Johnson, NJ
Melissa Przyborowski, Biomedical Engineering	Scientific Support Specialist, PromoCell, US
Renea Faulknor, Biomedical Engineering	Manager, Global Regulatory CMC Biologies, Celgene, NJ
Mehdi Ghodbane, Biomedical Engineering	Investigator, GlaxoSmithKline, PA
Andrea Gray, Biomedical Engineering	Biomedical Engineer, Food and Drug Administration, MD
Kristina Hernandez, Neuroscience	Medical Writer, Meditech Media, NY
Oleg Milberg, Biochemical Engineering	Scientist, Janssen Pharmaceuticals
Jillian Nguyen, Neuroscience	Technical Analyst, Cornerstone Macro New York, NY
Phil Tedeschi, Pharmacology	Senior Scientist, Pfizer, NJ
Gabriel Yarmush, Biomedical Engineering	Research Associate, Biomedical Engineering, Rutgers, NJ
Perry Yin, Biomedical Engineering	Consultant Analyst, PA Consulting Group, New York, NY
Elizabeth Stucky, Chemical and Biochemical Eng	Postdoctoral Research Associate, Manchester Institute of Biotechnology, UK
Brittany Taylor, Biomedical Engineering	Vice Provost Postdoctoral Research Fellow, Orthopedic Research Laboratories, University of Pennsylvania
Agnes Yeboah, Chemical and Biochemical Eng	Senior Director, Regulatory CMC Biologics and Cell Therapy, Celgene, NJ
Jake Jacobs, Biology and Biochemistry	Manager, Microbiologist, Troy Corporation, Florham Park, NY
Alvin Chen, Biomedical Engineering	Research Scientist, Phillips Healthcare, Cambridge, MA
Ana Rodriguez, Biomedical Engineering	Senior Medical Writer, BIONYC, NY

BIOTECHNOLOGY TRAINING PROGRAM ALUMNI

Ana Gomez, Biomedical Engineering	Senior Quality Engineer, Salvona Technologies, NJ
Kate O'Neil, Biomedical Engineering	Postdoctoral Fellow, University of Maryland, College Park
Kathryn Drzewiecki, Biomedical Engineering	AIMBE Scholar, FDA, MD
Trevan Locke, Chemical and Biochemical Engineering	Scientific Program Administrator, American Assoc for Cancer Research, Washington DC
Sal Ghodbane, Biomedical Engineering	R&D Formulating Scientist, Ethicon, NJ
Chris Lowe, Biomedical Engineering	Senior Upstream Development Engineer at Takeda Pharma, MA
Corina White, Biomedical Engineering	Process Engineer at Amicus Therapeutics, NJ
Seul-A Bae, Biomedical Engineering	Postdoctoral Fellow at Merck, NJ
Sarah Misenko. Biochemistry	Global Medical Affairs Oncology. Investigator Bio Asset Mgt. GSK, PA
Dharm Patel, Biochemistry	Project Manager, Medical Strategy and Scientific Affairs, LEO Pharma, NJ
Antoinette Nelson, Biomedical Engineering	Legislative and Policy Intern – US Senate Budget Committee
Daniel Browe, Biomedical Engineering	Postdoctoral Fellow, Harvard Medical School, MA
Jose James, Biomedical Engineering	Medical Student, RWJMS
Przemyslaw Swiatkowski, Cell Bio and Neuroscience	Postdoctoral Fellow University of Pennsylvania, School of Medicine
Paulina Krzyszczyk, Biomedical Engineering	Scientific Writer, Bristol Myers Squibb
Jeremy Anderson, Biomedical Engineering	Associate Scientist, Molecular and Analytical Development, Bristol Myers Squibb
Alison Acevedo, Chemical Engineering	Computational Biologiest, GSK
Jenna Newman, Molecular Biosciences	Postdoctural Fellow, Dana-Farber Cancer Institute, Harvard Medical School
Eve Reilly, Molecular Biosciences	Associate Consultant, Cello Health Bioconsulting
Misaal, Patel, Biomedical Engineering	Bioprocess Intern, Merck