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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 2016-17 year marks the 27th 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 2017-18 year, the NIH is providing 9 fellow positions and the University is providing an additional 5 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 by 2015.

**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.
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.

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.

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.

**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.

**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.

**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.

**Summer Industrial Internship Program - Appendix E**

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.

**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

<table>
<thead>
<tr>
<th>Faculty</th>
<th>Role in Program</th>
<th>Research Interest</th>
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| Androulakis, Ioannis, PhD  
Associate Professor  
Biomedical Engineering | Mentor | Systems biology, transcription, inflammation |
| Arnold, Edward, PhD  
Professor  
Chemistry and Chemical Biology | Mentor | HIV, AIDS, drugs, vaccines, structural biology |
| Berman, Helen, PhD  
Board of Governors Prof  
Chemistry and Chemical Biology | Mentor | Structural biology, structural bioinformatics |
| Berthiaume, Francois, PhD  
Associate Professor  
Biomedical Engineering | Executive Committee | Regenerative med, metabolic eng, stem cells for skin wounds |
| Bertino, Joseph, MD, PhD  
Professor  
Pharmacology | Mentor | Tumor suppressor genes and drug resistance |
| Bunting, Sam, PhD  
Assistant Professor  
Molecular Biology and Biochemistry | Mentor | Cell survival and DNA repair in mammals |
| Burley, Stephen, MD, PhD  
Distinguished Professor  
Chemistry and Chemical Biology | Mentor | Structural biology and proteomics |
| Cai, Li, PhD  
Associate Professor  
Biomedical Engineering | Mentor | Tissue engineering, stem cells, retinal cells |
| Copeland, Paul, PhD  
Associate Professor  
Biochemistry & Molecular Biology | Mentor | Regulation of gene expression at the translational level |
| Drake, Justin, PhD  
Assistant Professor  
Medical Oncology | Mentor | Cancer metastasis, Kinase signaling, Targeted therapies  
Targeted therapies |
| Dunn, Michael, PhD  
Associate Professor  
Orthopaedic Surgery | Mentor | Musculoskeletal Tissue engineering |
| Ebright, Richard, PhD  
Board of Governors Prof  
Chemistry and Chemical Biology | Mentor | Transcription; Antibacterial  
Drug Discovery |
| Firestein, Bonnie, PhD  
Professor  
Cell Biology and Neuroscience | Mentor | Dendrite branching in forebrain and spinal cord neurons |
| Freeman, Joseph, PhD  
Associate Professor  
Biomedical Engineering | Mentor | Repair of musculoskeletal tissues; tissue engineering |
| Gormley, Adam, PhD  
Assistant Professor  
Biomedical Engineering | Mentor | Bioinspired nanobiomaterials |
| Grumet, Martin, PhD  
Professor  
Cell Biology and Neuroscience | Executive Committee | Control of Inflammation after spinal cord injury |
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<tr>
<th>Faculty</th>
<th>Role in Program</th>
<th>Research Interest</th>
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| Ierapetritou, Marianthi, PhD  
Professor  
Chemical and Biochemical Engineering | Mentor | Systems engineering, metabolic engineering |
| Khare, Sagar, PhD  
Assistant Professor  
Chemistry and Chemical Biology | Mentor | Design principles of molecular recognition |
| Kramer, Sunita, PhD  
Associate Professor  
Pathology & Laboratory Medicine | Mentor | Cell migration, signaling, heart and blood vessel development |
| Lee, Ki Bum, PhD  
Associate Professor  
Chemistry and Chemical Biology | Mentor | Nanomedicine and Controlling stem cell/cancer fate |
| Lobel, Peter, PhD  
Professor  
Pharmacology | Executive Committee | Hereditary neurodegenerative diseases, functional genomics |
| Madura, Kiran, PhD  
Professor  
Pharmacology | Mentor | Ubiquitin-mediated protein degradation |
| Marcotrigiano, Joseph, PhD  
Associate Professor  
Chemistry and Chemical Biology | Executive Committee | Structure and function of hepatitis C viral proteins |
| Messing, Joachim, PhD  
University Professor  
Genetics | Mentor | Molecular biology of plant development |
| Millonig, James, PhD  
Associate Professor  
Neuroscience & Cell Biology | Mentor | Neurodevelopmental disorder |
| Moghe, Prabhas, PhD  
Professor  
Biomedical Engineering | Mentor | Stem cells, nanobiomaterials, tissue engineering |
| Montelione, Gaetano, PhD  
Distinguished Professor  
Molecular Biology and Biochemistry | Mentor | Bioinformatics / hybrid structure determination methods |
| Muzzio, Fernando, PhD  
Professor  
Chemical and Biochemical Engineering | Mentor | Pharmaceutical engineering; chaos & mixing |
| Nanda, Vikas, PhD  
Associate Professor  
Biochemistry & Molecular Biology | Mentor | Protein evolution and folding, de novo design of proteins |
| Olabisi, Ronke, PhD  
Assistant Professor  
Biomedical Engineering | Mentor | Tissue eng, regenerative medicine for injury and disease |
| Parekkadan, Biju  
Associate Professor  
Biomedical Engineering | Mentor | Develops platform technologies for cell and gene therapy |
| Pedersen, Henrik, PhD  
Professor  
Chemical and Biochemical Engineering | Executive Committee | Plant cell culture, chemical and biochemical fiber optic sensors |
| Roth, Charles, PhD  
Professor  
Biomedical Engineering | Mentor | Nucleic acid delivery, nanobiotechnology, cancer |
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<tr>
<th>Faculty</th>
<th>Role in Program</th>
<th>Research Interest</th>
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<tr>
<td>Shreiber, David, PhD</td>
<td>Mentor</td>
<td>Nervous system repair, biomechanics, tissue eng</td>
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<td>Associate Professor Biomedical Engineering</td>
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<td>Sinko, Patrick, PhD</td>
<td>Mentor</td>
<td>Biopharmaceutics; intestinal absorption; peptide drugs</td>
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<td>Pharmaceutical Sciences</td>
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<td>Sofou, Stavroula, PhD</td>
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<td>Biomembranes, drug delivery, tissue engineering</td>
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<td>Stock, Ann, PhD</td>
<td>Co-Director</td>
<td>Structure/function analysis of signal transduction proteins</td>
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<td>Professor Biochemistry &amp; Molecular Biology</td>
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<td>Sy, Jay</td>
<td>Mentor</td>
<td>Applying biomaterials chemistry to prototype medical devices.</td>
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<td>Welsh, William, PhD</td>
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<td>Drug discovery, computer-aided modeling and design</td>
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<td>White, Eileen, PhD</td>
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<td>Oncogenes, tumor suppressor genes, apoptosis, autophagy</td>
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<td>Williams, Lawrence, PhD</td>
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<td>Molecular structure and reactivity.</td>
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<td>Yarmush, Martin, MD, PhD</td>
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<td>Zaratiegui, Mikel, PhD</td>
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<td>Chromatin dynamics, transposons, silencing</td>
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<td>Mentor</td>
<td>Tumor immunology, combination cancer immunotherapy, viral infections</td>
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<td>Assistant Professor Medical Oncology</td>
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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, while focusing on MPL response in the liver, has a broader aim: 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 two aims: (Specific Aim 1 - completed). In aim 1, we used combined genomic and liver-specific transcriptomic and proteomic data from ADX male rats following MPL dosing to generate gene-protein interaction networks. Specifically, we identified quantitative relations within high-throughput, time-varying, MPL-regulated mRNA and proteins, in order to identify putative regulatory links between the two and enable network reconstruction. (Specific Aim 2 – in progress) In aim 2, we will focus on characterizing the temporal dynamics of liver-specific gene-protein interaction network following MPL dosing in ADX male rats. 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.

PRESENTATIONS


JEREMY ANDERSON
Advisor: Li Cai

Traumatic brain injury (TBI), defined as a mild or 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 can aid in the development of novel therapeutics promoting neurogenesis and functional recovery.

Our 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, to characterize the activation of endogenous NSCs after TBI to determine their potential in injury repair and neural regeneration, will be accomplished through the implementation of the following specific aims: 1) to refine a closed head injury (CHI) model for use in our studies, 2) to characterize GFP+ NSC identity, proliferation, migration, activation, and fate post-TBI, 3) to identify genes highly active in NSCs after injury (using e.g., qPCR, single-cell RNA-seq), and 4) to validate these genes in vitro and in vivo.

To date, we refined our CHI model, and characterized the GFP+ NSCs after injury in this model. Transgenic mice 8-12 weeks of age were injured using the CHI model. The mice were analyzed 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. Immunohistochemistry identified that GFP+ NSCs are expressed in higher levels after injury compared to control/sham animals with a peak at 2 days post injury (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).

We currently are pursuing aim 3 to identify genes activated in NSCs after TBI compared to uninjured NSCs using qPCR and single-cell RNA-seq to identify unique genes activated after brain injury. Upon identification of upregulated genes in injured NSCs, we will validate these genes using overexpression and knockout assay in vitro. Understanding the functions of these genes will identify important genes and pathways crucial to cellular response after TBI. Understanding the characteristic activation of NSCs after TBI and its associated genes will help elucidate the transcriptome changes induced by TBI, to determine NSC potential in injury repair and neural regeneration.

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SEUL-A-BAE
Advisor: Ioannis Androulakis

Food availability and intake is a strong environmental signal that can entrain the biological rhythms of the periphery along with the light/dark cycle. Recent studies revealed that nutrient availability has close ties to circadian rhythms, exhibiting bi-directional influence and complex signaling cascades. In mammals, metabolic activities are under the regulation of daily feeding rhythms as well as the peripheral clock machinery. In turn, the feeding rhythms influence the circadian rhythms of key clock components via enzymatic reactions and transcriptional regulation. As a result, circadian disruption is linked to clinically significant metabolic abnormalities such as diabetes mellitus, obesity, and high level of tri-glycerides. On the other hand, time restricted feeding (TRF) has been shown to be effective in restoring the circadian rhythmicity, also deterring disease progression. Therefore, it is imperative to understand the mechanism behind how meal timing in relation to the light/dark cycle affects the host’s disease.

In humans, the light/dark cycle synchronizes the rhythms of the peripheral tissues by entraining the release of cortisol from the hypothalamic-pituitary-adrenal (HPA) axis. In the periphery, cortisol interacts with the glucocorticoid response element (GRE) to regulate the transcription of clock genes such as Per and Cry, whose robust rhythms are necessary for healthy metabolic functions. The objective of my project is to understand the role of feeding cycle in the neuroendocrine system and its phase relations to the light/dark cycle entrainment.
via a mathematical model. Last year, I hypothesized that a feeding-induced increase in the NAD$^+$ level inhibits SIRT1, which regulates some of the components in the core clock machinery such as the PER/CRY and CLOCK/BMAL1 protein complexes via deacetylation. Mathematical modeling of this network showed that the feeding cycle can independently entrain the clock genes in the periphery, and revealed that there exists an optimal phase relationship between the light/dark cycle and feeding cycle for robust expression of circadian rhythmicity. This year, I selected hepatic gluconeogenesis as a representative metabolic reaction that is entrained by both of light/dark and feeding cycles, and am currently investigating the entrainment dynamics via the previously modeled neuroendocrine components and SIRT1. My hypothesis is that the dynamics of gluconeogenic genes such as $Pck1$ and $G6pc$ are regulated by the convoluting effects of cortisol and SIRT1 through the key elements like PGC-1α and FOXO1. I expect to observe deterioration in rhythmicity of gluconeogenic gene expression under abolition of oscillation in light or feeding. Upon completion, this work will provide insight into the mechanism of how circadian disruption results in abnormal metabolism as well as the functions of peripheral clock genes in metabolic diseases.

**PRESENTATIONS**


**PATENTS**


**AWARDS**

NSF Graduate Research Fellowship Program 2015

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**DANIEL BROWE**

Advisor: Joseph Freeman

There are about 1.6 million individuals in the United States that have had a limb amputation due to a traumatic injury, and there are roughly 80,000 new amputations each year. In many cases, the limb must be removed due to significant skeletal muscle/peripheral nerve loss despite little or no bone loss. Tissue engineering strategies to regenerate large voids in skeletal muscle may be able to prevent limb amputation for certain patients. Current tissue engineering strategies typically involve seeding myoblasts or muscle progenitor cells on some sort of biomaterial scaffold; however, this approach fails to generate mature skeletal muscle tissue with adequate contractile strength. Although researchers have successfully recreated the conditions for myoblasts to fuse into myotubes, or immature muscle fibers, there remains a gap in the knowledge on how to induce these myotubes to further differentiate into muscle fibers capable of producing forceful contractions.

Artificial muscles have the capability to restore normal force production and motion to damaged limbs by augmenting force production in a design that resembles an exoskeleton. A group of “soft” actuators including electroactive polymers and ionic polymer-metal composites have the ability to produce comparable contractile stresses to native muscle tissue while remaining relatively lightweight. The disadvantages of artificial muscles are that they typically require some sort of power source, and they don’t allow for restoration of muscle tissue when used externally, which may necessitate eventual limb amputation. The
Objective of this project is to develop a biocompatible artificial muscle for skeletal muscle regeneration that will provide electrical and mechanical stimulation to developing myoblasts. This contractile, composite scaffold seeks to closely mimic the in vivo environment of developing skeletal muscle, producing highly organized and differentiated muscle tissue. The central hypothesis of this project is that the mechanism of movement in an ionic electroactive polymer and can be used to achieve the speed and strength of contraction found in native muscle tissue, that these movements can be precisely controlled by electrical stimulation with high repeatability, and that the resulting electrical and mechanical stimulation will provide an environment for muscle cells that encourages growth and differentiation into organized muscle fibers.

Many studies have previously shown the beneficial effects of both electrical and mechanical stimulation on myoblast growth, differentiation, and organization. Also, our preliminary findings with a scaffold that resembles an ionic polymer-metal composite confirm that scaffold contraction is possible using a hydrogel made of poly(ethylene glycol) (PEG) and poly(acrylic acid) (PAA) and a fibrous scaffold made of polycaprolactone (PCL) and polypyrrole (PPy). We plan to test our hypotheses through the pursuit of the following specific aims: 1) to develop, characterize, and evaluate the ability of a conductive, nanofibrous scaffold made of PCL and PPy to promote the organization and differentiation of myoblasts into myotubes; 2) to develop and characterize a biocompatible, electroactive hydrogel made of PEG and PAA which actuates in an electric field; 3) to characterize the in vitro response and evaluate the effect on myoblast differentiation of combined electrical and mechanical stimulation provided by the contractile, composite scaffold; and 4) to evaluate the in vivo response to the developed contractile, composite scaffold in a small animal model.

Previous studies have shown that myoblasts respond favorably when seeded on fibrous scaffolds made of conductive materials. Myoblasts respond to both topographical cues, in the form of aligned fibers, and passive electrical cues, in the form of a conductive material, by fusing into myotubes in the same direction as the alignment of scaffold fibers. We have completed aim 1 of this project by synthesizing a copolymer of PPy, a conductive and biocompatible polymer, and PCL, a biocompatible polymer with favorable mechanical properties, to design a conductive fibrous scaffold for myoblast growth and development. Once synthesized, the PPy-PCL copolymer was fabricated into a fibrous mesh using a technique called electrospinning. When comparing the conductive PPy-PCL scaffold with a nonconductive PCL scaffold, we have found that the PPy-PCL scaffold promotes higher myoblast proliferation, fusion, and differentiation than scaffolds with PCL alone. Future studies will attempt to further correlate PPy-PCL scaffold conductivity with myoblast cell response.

Previous studies with electroactive polymers have extensively characterized a variety of materials that will actuate in an electric field. For aim 2 of this project, we developed a biocompatible, electroactive hydrogel made of PEG and PAA and characterized its movement in an electric field and the cellular response when seeded with myoblasts. When evaluating hydrogel movement, we identified three key factors which impact the speed and extent of movement. These factors are the geometry of the hydrogel (thickness to length ratio), the ratio of PAA to PEG, and the overall polymer concentration of the hydrogel. After testing a range of values for all of these parameters, we determined the optimal thickness to length ratio, ratio of PAA to PEG, and overall polymer concentration to produce the most movement in an electric field. When seeding myoblasts on these hydrogels, we discovered that cell attachment greatly depended on the ratio of PEG to PAA. With very low concentrations of PAA, the cells failed to adequately attach to the hydrogels, and with very high concentrations of PAA, the acidity of the resulting solution resulted in high cell death. We found that a ratio of about 4:1 of PEG:PAA was ideal for cell attachment. However, the myoblasts failed to differentiate fully on any hydrogel sample.

Future work in this project will combine the materials developed in aims 1 and 2 in order to produce a composite scaffold that will support myoblast attachment, growth, and differentiation, while also allowing us to test our central hypothesis about applying simultaneous electrical and mechanical stimulation to developing myoblasts. These tests will utilize a range of stimulation patterns with different amplitudes and
frequencies of applied voltage to discover the optimal pattern of stimulation. Finally, the composite scaffold will be evaluated in a small animal model to determine the effect on live tissue.

**PRESENTATIONS**


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**LARRY CHENG**  
Adviser: Justin Drake

Metastatic castration-resistant prostate cancer is a lethal disease with few available treatment options. Many kinase inhibitors are available on the market that are used for the treatment of cancer, but none are approved for prostate cancer. The challenge is that there are currently no therapeutic strata or biomarkers available to guide prostate cancer treatment. Our strategy is to utilize phosphoproteomic approaches to observe changes in pathway signaling as a means to identify novel targets for therapy as well as biomarkers to stratify and predict response to current kinase inhibitor therapy. Since joining the Drake laboratory, I have worked to build up the downstream computational and statistical components of our lab’s shotgun phosphoproteomic pipeline. The purpose of the computational pipeline is to streamline our data processing and information gathering. We use the MaxQuant software to predict phosphopeptide sequences and calculate their intensity values from the mass spectrometry raw data. The pipeline applies filters to remove poorly predicted phosphopeptides as well as phosphopeptides that are statistically insignificant between samples. The remaining phosphopeptides are mapped to protein sequences in the UniProt database, searched against PhosphoSite for functional annotations of their phosphoresidues, and mapped to upstream kinase motifs from databases including PhosphoSite, NetworKIN, Phosida, and the Human Protein Reference Database. The pipeline generates a tidy file that is allowable for manual inspection as well as usable to perform downstream analyses. The analysis tools that I have streamlined for our lab up to this point include unsupervised and supervised hierarchical clustering, kinase-substrate enrichment analysis, the DAVID functional annotation tool, and gene-set enrichment analysis. While establishing this infrastructure, I have already utilized the pipeline and downstream tools to analyze the phosphoproteomic data for several collaborators.

The next phase of my work is the upstream assay development for our targeted proteomics approach. Although routinely performed tests generate enormous information regarding genomic aberrations about patients in the clinic, proteins are the ultimate effectors of biological function. I am currently in the process of designing peptides for 52 select genes of interest that are in common between the Cancer Gene Mutation Panel 50 assay (CGMP_50), the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), and FoundationOne’s DNA gene list. These peptides will serve as our internal standards for precise quantitation of protein expression. If this assay can be robustly developed and clinically validated, it may help bridge this gap to improve clinical diagnostics and therapy selection.

**AWARDS**

Proteomics Interdisciplinary Research Award, Institute of Quantitative Biomedicine 2016  
Joint PhD Excellence Award, Institute of Quantitative Biomedicine 2016
The overall goal of my thesis is to study cancer organoids for use in developing personalized medicine strategies. Organoids are three-dimensional (3D) in vitro models of organs or tissue derived, in many cases, from a single stem cell. Because tumors contain stem cells, personalized organoids can be created to model a patient’s individual tumor morphology and makeup. Prostate cancer is an attractive model for organoid development due to its lack of available cell lines, as well as the prevalence of the disease. However, researchers growing prostate cancer organoids (PCOs) have encountered issues in growth efficiency and longevity. I hope to study the complex 3D biological interactions between the cell and non-cell components of the organoids to help resolve many of the current organoid challenges.

While the goals of the organoid project are being developed, I have worked on a secondary project that focuses on creating a co-treatment of local anesthetics and mesenchymal stromal cells (MSCs). This secondary project has allowed me to explore the connection between cells and non-cell materials, both experimentally and computationally. The skills and techniques I have learned from the anesthesia project will presumably help me understand the dynamics of a large organoid 3D model system. Local anesthetics (LA) act by blocking sodium channels, which leads to the prevention of sensory pain receptors relaying information to the brain. Administration of LA is a commonly employed practice in many procedures to minimize patient surgical and post-surgical pain. MSC therapies have been used for a variety of tissue regenerative and inflammatory applications and are often co-administered with LA; however, LA can affect MSC viability and function. Previous research conducted in the Yarmush lab has shown that in the presence of LA, MSC cell secretions and the MSC ability to induce macrophage anti-inflammatory responses were altered. Therefore, finding an improved method to co-administer LA with MSC cells is necessary.

Recently, we 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. COMSOL was used to model the release profile of LA from the hydrogel-liposome system to ensure that the concentration of drug that the MSCs are exposed to, or the cell-apparent concentration, remains low. Previous work indicated that a cell-apparent concentration of 0.1M enables a 90% cell viability. Our model indicated that the hydrogel-liposome system, with an initial dose of 1M, did not exceed a cell-apparent concentration of 0.1M, and that drug release was maintained for 4 days. In vitro analysis has shown that there is a significant increase in MSC cell viability when the hydrogel construct is applied compared to a bolus dose or the bupivacaine-containing liposome alone. Cell secretion analysis to ensure functionality of MSC is currently being studied. We are also analyzing how the alginate-liposome construct affects MSCs that have been stimulated to mimic an inflammatory environment, as well as the interactions within a co-culture system containing both MSCs and macrophages. Some of these findings have been published, and a second manuscript is in progress, which will focus on the current in vitro functionality findings.

PRESENTATIONS

The path to tissue malignancy is often presaged by a variety of mutations. Present in a variety of cancers, one of the most common is the mutation of p53, a tumor suppressor protein. This mutation will often cause overexpression of the mutant protein, which then leaks out of the cytoplasm, resulting in the production of auto-antibodies to p53 in many cases. While one study found that p53 autoantibodies were detectable in 26% of current and future colon cancer patients using a custom made immunoassay, their methodology for detecting p53 antigens was flawed in that it did not account for mutant versus native protein effects. In addition, we question whether the assay was sufficiently sensitive to detect low levels of polyclonal antibodies that may have been present in those counted as negative by their methodology. We hope to improve upon their results by utilizing a combination of microfluidics and a novel protein energetics model in order to create an ultrasensitive assay that could potentially be applied to p53 and other diagnostic biomarkers.

In my first year at Rutgers I read relevant literature, learned the theory behind the protein model, and practiced the laboratory techniques I would be using for the experimental assay portion. This year, I intend to complete conventional plate immunoassays in order to see how results from those compare to the data from an unoptimized microfluidic device which was developed by a previous graduate student in Dr. Yarmush’s lab. I also want to improve upon the current device and its operation, by finding a way to reduce its relatively long washing times and by adding analysis capability on-chip, such as with built-in optics. Finally, I intend to begin selecting and synthesizing the optimal p53 antigens (those that would be most expressed and targeted in vivo) using our Excel-based program. I am currently taking a protein synthesis and purification class to help me with the latter part of this task.

SAL GHODBANE
Advisor: Michael Dunn

The menisci are semilunar fibrocartilaginous disks, which play several important roles within the tibiofemoral joint including shock absorption, force transmission, load distribution, joint stability, and lubrication. Partial meniscectomy is currently the gold standard for treatment of meniscal lesions within the inner 80% of the meniscus. Being the most common orthopedic procedure, partial meniscectomies cost upwards of $4 billion per annum. The meniscus’ function stems from its chemical and physical composition; most importantly, a complex network of collagen fibers allows the meniscus to convert compressive axial loads into circumferential tensile loads. Therefore, it is not surprising that there has been a well-documented correlation between meniscectomy volume and osteoarthritis due to changes in the normal mechanical loading. This has led to a trend towards meniscal tissue engineering as an avenue to regenerate meniscal tissue instead of simply removing it. An ideal meniscus scaffold would be of an appropriate geometry to fit into the replacement site, facilitate cell attachment, infiltration, and distribution, and possess appropriate mechanical properties to stimulate the regeneration of meniscal tissue and protect the underlying bone surfaces. 3D printing is a method of manufacturing in which materials are deposited onto one another in layers to produce three-dimensional structures. This method allows for the fabrication of complex and interconnected architectures needed to mimic the geometry of the native meniscus. The objective of this study is to develop an acellular scaffold that can be trimmed to appropriate defect geometries for partial meniscus regeneration. The central hypothesis is a 3D printed polymer-reinforced collagenous scaffold can be fabricated and implanted to: 1) mimic the mechanical anisotropy of the native meniscus, 2) be successfully fixed to the remaining native meniscal rim, 3) increase the contact area and reduce peak stresses relative to partial meniscectomy, 4) encourage cell infiltration, extracellular matrix production, and organized tissue deposition, 5) integrate robustly to the surrounding native meniscal tissue, and 6) protect the articular
surfaces to prevent or delay degenerative changes in the underlying cartilage. Our hypothesis has been formulated based on our lab’s previous experience with a total meniscus implant, which has had significant success in large animal studies. The rationale of this study is that by mimicking the anisotropy of the native meniscus, infiltrating cells will be provided with the correct mechanical stimuli to lead to fibrocartilage tissue formation. We plan to test our hypothesis through the pursuit of the following specific aims: 1) determine the mechanical and enzymatic differences among bovine, porcine, and ovine derived collagen scaffolds to identify a source for the collagen, 2) optimize the mechanics of the 3D printed polymer architecture to approach the anisotropic tensile mechanics of the native meniscus, 3) develop a surgical procedure that will produce suitable fixation that allows for tissue ingrowth and integration and improved functional biomechanics than simply suture techniques, and 4) evaluate the regenerative properties of the implant through a functional in vivo in an ovine posterior partial meniscectomy model.

MADISON GODESKY
Advisor: David Shreiber

Hyaluronic acid (HA) is a ubiquitous extracellular matrix glycosaminoglycan responsible for diverse regulatory functions within connective, epithelial, and neural tissues. HA is an attractive biomaterial for soft tissue regeneration because it presents physicochemical properties that mirror native soft tissue, including high water content and excellent permeability for dissolved oxygen, nutrients, and metabolites. Due to its polyanionic nature, pure HA inhibits cellular attachment; so as a substrate for tissue engineering, this characteristic must be overcome. However, this property also presents an opportunity to design selectively-permissive biomaterials by functionalizing HA with specific peptide-ligand sequences. For example, in applications prone to post-implantation scarring, selectively-permissive materials hold the potential to permit cells of interest to grow while limiting immune cell or fibroblast infiltration into the tissue scaffold.

One HA-based biomaterial that is available commercially and has been applied to diverse applications in regenerative medicine is the thiol-modified carboxymethyl hyaluronic acid (CMHAS) and poly(ethylene) glycol diacrylate (PEGDA) hydrogel. The mechanical properties of CMHAS-PEGDA hydrogels are unique because two reactions, occurring at discrete time points, contribute to the steady-state crosslinking density of the network. We hypothesize that grafting bioactive ligands to CMHAS-PEGDA gels can selectively interrupt latent disulfide bond formation, a spontaneous process that dramatically stiffens the material over a period of days to weeks. Native extracellular matrix is not a continuum of physicochemical properties, but a dynamic network in which both mechanical stiffness and adhesion ligand density regulate cell phenotype. Therefore, we aim to independently manipulate stiffness and ligand density in CMHAS-PEGDA hydrogels to control cell fate.

Using rheology, we characterized the mechanical properties of CMHAS-PEGDA hydrogels and demonstrated that the elastic modulus can be manipulated by targeting either rapid (thiol-acrylate) or latent (thiol-thiol) crosslinking reactions. Moreover, we also determined that the latent disulfide crosslinking reaction provides a greater range to control the steady-state mechanical properties. Targeting this latent stiffening reaction, we grafted acrylate-functionalized ‘mock ligands’ to CMHAS-PEGDA gels to block latent crosslinking. Using both rheological and spectral methods, we determined that acrylate-functionalized molecules covalently graft to remaining free thiols on the CMHAS backbone and inhibit latent stiffening reactions to controllable degrees. When acrylate-functionalized cell adhesion ligands (acryl-GRGDS peptides) were used in place of the ‘mock’ molecules to block latent crosslinking, we observed a significant increase in cell attachment to the gels (2597 fibroblasts/cm2). When a control GRGDS peptide lacking the acrylate functional group was added, almost no cell attachment (3 cells/cm2) was observed.
We have determined that the unique crosslinking properties of CMHAS-PEGDA hydrogels can be used to co-modulate matrix stiffness and adhesion ligand density. Current work involves independently tuning these properties to present patterns, including gradients, which influence cell fate in native tissues. We plan to use CMHAS-PEGDA hydrogels as selectively-permissive substrates for tissue engineering with patterned, biomimetic signals to regulate cell fate. Potential applications for future work include regulating migration-based processes such as embryonic development, cancer cell metastasis, or peripheral nerve regeneration.

PRESENTATIONS


Godesky M, Shreiber D. Hyaluronic Acid-Based Hydrogels with Simultaneously Tunable Mechanical and Bioactive Properties. 2016 Biomedical Engineering Society Annual Meeting, Minneapolis, MN. October 2016.

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. The neurons that we study are embedded in the C. elegans hypodermis, which may play a role in exopher-genesis. To address the question of whether genes identified in previous screens are functioning in a cell-autonomous or
non-cell-autonomous manner, I am creating a strain that expresses sid-1 (the receptor protein necessary for RNAi uptake) exclusively in hypodermal tissue.

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.

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 more quickly when viewed in Petri dishes under low magnification fluorescence dissecting microscopes. 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. Toward making such a device, I have enrolled in a BME microfluidics course taught by Dr. Jeffrey Zahn.

AWARDS

AAAS/Science Program for Excellence in Science May 2016

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ALAINA HOWE
Advisor: Stavroula Sofou

Often after surgery to remove tumors in ovarian cancer patients, it is not clear if all malignant cells have been eliminated; this creates a risk for patients being sent home with lingering cancer cells. Currently, there is no test for these residual cells and oncologists are left to either administer an extraneous round of treatment or release a patient with some uncertainty. It is hypothesized that an injection of folate receptor targeting liposomes, carrying imaging agents, in the peritoneal cavity, could detect residual cancer cells; this would allow oncologists to administer additional treatment only if necessary. Folate receptor α (FR-α) is overexpressed in most ovarian cancer patients, and is under expressed in healthy tissue, making it a prime receptor for selective targeting. However, in cases where FR-α has medium or low expression, current targeting methods are not particularly effective. It has been previously shown that liposomes can be formed
to have pH-dependent “sticky patches” which have higher binding than those with homogenously distributed targeting ligands. Using “sticky” liposome carrying imaging agents could allow detection of residual cancer cells, and allow oncologists to administer additional treatment with greater discretion.

To date, I have performed several binding and internalization studies to assess the binding of uniformly targeted liposome conditions to monolayers of the FR-α overexpressing ovarian cancer cell line SKOV-3. Moving forward, I intend to evaluate liposome preparations with “sticky patches” on ovarian cancer cell lines with varying levels of FR-α expression. My short term goal is to design a sticky targeted liposome that can effectively bind to cell lines with a range of expressions. Once this is achieved, I can scale to spheroids, an in vitro tumor analog, and animal studies.

In addition, I have been studying phase separation of lipids in E. coli plasma membranes through the use of giant unilamellar vesicles (GUVs). E. coli plasma membranes are composed of PE and PG lipids with a range of tail lengths. It has been shown that phase separation occurs in the membranes, along which MreB polymerization also occurs. In an effort to further understand the mechanism, I have created and imaged several lipid compositions to observe domain formation in GUVs. Currently, I have been attempting to determine the composition of the domains using nonyl acridine orange (NAO), a dye shown to selectively bind to PG lipids in E. coli cells. This could give insight into the mechanism of MreB polymerization, in which phase separated domains act as a template and polymerization occurs along the interfaces.

Finally, I am also involved in a project aimed at understanding the mechanism of action of a specific antibiotic. Daptomycin is an antibiotic used for treatment of antibiotic-resistant gram positive bacterial strains, and while there are several theories for the cell membrane disrupting mechanism of daptomycin action, the detailed mechanism is not currently known. It has been shown that daptomycin is more effective in gel-like membranes than fluid membranes, and our hypothesis is that registration of domains between leaflets in the gel phase allows for interfaces to form, along which daptomycin is able to act. However, in fluid phases the undulating tails do not allow for daptomycin to effectively insert itself along the phase separated domains. To prove this, we propose creating asymmetric liposomes in which the outer leaflet is composed of a gel membrane and the inner leaflet is composed of a fluid membrane. The expectation is that without registration of gel domains on both leaflets, daptomycin will not be effective. I have successfully created asymmetric liposomes and have begun to work with relevant compositions and study the effect of daptomycin

JAMES JOSE
Advisor: Vikas Nanda

Tropomyosin (Tpm) is a protein involved in the regulation of the cell cytoskeleton. Minor sequence variations produce dramatic effects on Tpm folding resulting in diseases such as Familial Hypertrophic Cardiomyopathy (FHC). These clinical outcomes demonstrate a need to understand and model accurately how key residues globally impact Tpm structural stability and dynamics. The structure of Tpm is a coiled-coil homodimer, where all direct residue contacts are local in sequence. Tpm deviates significantly from the ideal knob-in-hole packing for coiled-coils due to the presence of stabilizing and destabilizing clusters 1-2. As evidence of Tpm’s non-ideal structure, thermal denaturation displays multiple transitions corresponding to semi-independent cooperative blocks 3. Unlike the classical two state models for protein folding, the multiple transitions observed in the Tpm melt suggest a more gradual unwinding, where different regions exhibit a local folding transition apart from the rest of the protein. However, the exact size or the nature of the boundaries that demarcate regional folding is unknown due to the continuous coiled-coil structure. This is in contrast to other proteins that display multiple unfolding transitions such as fibronectin, whose transitions correspond to distinct globular subunits in a chain connected by an unstructured linker region acting as a boundary. Whether these cooperative folding units
seen from unfolding experiments at equilibrium conditions reflect a kinetic pathway is uncertain, rather they represent the population of states exhibiting the lowest free energy for a temperature. Thus a folding domain in these and the following experiments reflects regions with similar or coupled conformational probabilities through intermediate equilibrium states.

Flexibility garnered from tuning of folding domains is essential for allosteric motions to regulate actin-myosin interactions. Single amino acid substitutions alter the relative stability between folding domains, impacting overall flexibility. A major step toward understanding the long-range impact of residue changes on folding is to develop a model for Tpm folding domains defined by sequence. We hypothesize that the dynamics of Tpm folding domain boundaries specify long-range stability and flexibility. By understanding how the boundaries between folding domains within Tpm are defined and how domains interrelate, we can create a model to explain how mutations can impact global folding and dynamics. Current progress on the following aims is described with the expectation that they will be completed for my thesis dissertation on May 2017.

Aim 1: To identify shifts in folding domains across distant homologs. We hypothesize that despite their high sequence similarity, vertebrate and invertebrate Tpm exhibit sufficient sequence differences to contrast in the stability of their folding domains. This hypothesis is inspired by multiple observations that invertebrate Tpm is a major food allergen and is known to be more resilient to gastric digestion, suggesting that vertebrate and invertebrate Tpm have differences in conformational stability. Choosing pig and shrimp homologs of Tpm as representative of these two classes, we assess the relationship between local and long-range stability with digestive resiliency between these homologs.

Current Progress: Multiple experiments have been conducted comparing the thermodynamic stability and digestive resilience of shrimp and pig Tpm homologs. These experiments consisted of using CD spectroscopy to measure helical conformation across a temperature melt of the entire protein as well as peptides derived from sequences spanning the coiled-coil. Digestive stability was measured by in vitro experiments replicating gastric and intestinal phases with semi-quantitative detection of cleaved peptides by LC-MS/MS. Comparing directly pig and shrimp Tpm revealed the latter to have more stable melting temperatures in both major transitions than the former. Simulated digestion has further shown slower kinetics of digestion in shrimp Tpm, particularly in a highly resistant C-terminal half. As regions with lower conformational stability are more susceptible to proteolysis, the increased stability seen by CD spectroscopy in shrimp corresponded well with its greater resilience to digestion. Molecular Dynamics (MD) provided further corroboration for the greater conformational rigidity in the shrimp homolog as pig Tpm was found to have larger long-range coiled-coil flexibility, especially within the middle region. These experiments revealed shrimp Tpm to be a more rigid and stable protein than the pig, with the middle region in particular providing the latter with its conformational flexibility. Observing a strong dichotomy between the N- and C-terminal halves of shrimp Tpm inspired an inter-regional comparison of stability and digestion. Semi-quantitative identification of peptide fragmentation found highly surviving peptides throughout shrimp sequence. However, measurement of helical conformation for a series of peptides spanning the protein indicated that only the peptides within the N-terminal half demonstrate significant local helicity, though these regions also corresponded to fragments with high survival in the digestion assays. Consequently, this suggests differences in peptide survival between the N- and C-terminal halves where the former depends on local stability, but the latter may benefit from long-range stability. This dichotomy further suggests the middle region to play an important role in separating these cooperative effects.

Aim 2: To define the boundaries of folding domains. We hypothesize that by outlining differences in the conformational dynamics along the coiled-coil structure we can demarcate regions with different overall stabilities. To more accurately assess local dynamics in relation to folding domains of the whole protein, we will use hydrogen deuterium exchange (HDX) over a temperature range that overlaps the folding transitions seen in CD, and measure exchange via mass spectrometry (HDX-MS). Since Tpm retains a consistent coiled-coil structure that is largely solvent exposed, amide exchange will primarily be reflective of backbone hydrogen bonding occupancy. In this manner we can measure local helicity in the context of the whole protein and determine which part of Tpm unfolds at a given transition.
Current Progress: Much work has been accomplished toward optimizing a method for HDX-MS in terms of identifying the ideal digestion, quenching, and chromatography conditions to maximize fragmentation but limit back exchange. Preliminary HDX data at low temperatures suggest the existence of three domains with the middle being the most dynamic, separating less exchanged or more stable N- and C-terminal regions. A temperature series HDX-MS run has been performed under a range that corresponds to a fully folded state, three transitions seen from melts with CD, and a fully unfolded state. Currently we are analyzing the data from this set of experiments by identifying regions with correlated exchanges across temperatures. However, in order to ensure the accuracy of our results, peptides are manually checked to make sure retention and m/z peaks are assigned properly across experiments for comparison.

Future Plans: HDX experiments will need to be repeated in order to ensure the robustness of our results. Additionally, corresponding MD trajectories will be run to determine if correlated dynamics correspond to HDX exchange rates. However, MD alone is likely not to be sufficient for modeling HDX due to insufficient sampling of the equilibrium conformation, as we expect HDX to be an equilibrium thermodynamic measurement. Consequently we recognize the importance of considering the Tpm ensemble as a whole when interpreting HDX data, and are actively developing a statistical model that may inform our interpretation of both HDX and MD data. With the completion of this aim we will be able, for the first time, to directly measure the region of Tpm that corresponds to a folding domain and provide insight to the relationship between sequence and domain boundaries.

Aim 3: To define boundaries of long-range correlated dynamics in Tpm. We hypothesize that specific regions or residues regulate global dynamics of Tpm by controlling long-range correlated motions. Since Tpm exhibits minimal contact order as a coiled-coil, long-range effects of single mutations must reflect changes in long-range correlated dynamics. By identifying correlated domains in Tpm we can determine the molecular origins of boundaries between domains.

Current Progress: MD trajectories have been produced up to 200 ns from different Tpm species, such as pig, rat, and shrimp with multiple types of single amino acid substitutions that have been shown in the literature to shift the melting temperatures of the major Tpm folding transitions and alter allosteric function. These trajectories revealed remarkably different behavior in global flexibility as measured by radius of gyration and end-to-end distances, or per residue dynamics by RMSF. In identifying correlated motions in MD we hope to explain these observed differences by understanding the coupling between high frequency local motions with long-range low frequency ones. We approached this by measuring covariance between residues as well as time correlations for a given residue. Using covariance we identified regions with correlated or anti-correlated fluctuations. Principal component analysis on these matrices produced eigenvalues and eigenvectors that correspond to a spectrum of low to high frequency modes. Corresponding eigenvectors to low frequency modes are studied to identify patterns of atomic displacement that may separate Tpm regions based upon collective motions. Time-correlation functions are used to compare atomic fluctuations across various time steps in a given residue’s simulated trajectory. Typically correlations exhibit a damping behavior as the correlated time interval gets larger; however, if low frequency nonlocal collective motions occur, we can observe them by oscillations superimposed on the damped function. Residues with similar superimposed oscillations share a domain defined by collective motion.

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**PAULINA KRZYSZCZYK**  
Advisor: Francois Berthiaume

Diabetes affects 29.1 million people in the United States, and non-healing ulcers are a major complication associated with the disease. In fact, diabetes is the leading cause of non-traumatic lower limb amputations, which are performed due to infected ulcers that fail to heal. Characteristics that prevent chronic diabetic wounds from healing include low oxygen and growth factor levels, and a persistent inflammatory state.
Current therapies, including even the most advanced ones, such as skin substitutes, are only effective 50% of the time. This may be due to the limited scope of many therapies, which target only a single deficiency of chronic wounds. We aim to develop a more effective chronic wound therapy that can promote characteristics found in healing wounds, such as increased levels of key growth factors, high oxygen levels, and the presence of M2 anti-inflammatory macrophages. To achieve this, we combine two components: alginate-encapsulated mesenchymal stem cells (MSCs) and polymerized hemoglobin (PolyHb). MSCs will serve to reintroduce growth factors to the wound, as well as promote the transition of pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages, which incidentally express increased levels of the hemoglobin (Hb) receptor CD163. CD163 endocytoses Hb, and activates the anti-inflammatory HO-1 pathway. Hb also has the ability to deliver oxygen to wounds. In our case, PolyHb will be used as a less toxic form of Hb, which can also be tuned to vary its biophysical properties and potential effects on macrophages. The aims will focus on: 1) investigating and optimizing the interactions between PolyHb, MSCs and macrophages, 2) developing a method to effectively deliver PolyHb/MSCs to diabetic wounds via sustained release of therapeutic factors, and 3) evaluating the effect of MSC/PolyHb combination therapy on the wound healing response and macrophage phenotype in an in vivo diabetic mouse model.

Recent progress on these aims is described as follows. In Aim 1, levels of secreted M1 and M2 macrophage markers, tumor necrosis factor-α (TNF-α) and interleukin 10 (IL-10), respectively, have been measured in macrophages exposed to various forms of PolyHb with or without haptoglobin (Hp), an acute phase protein that increases Hb-CD163 binding. Addition of Hp was shown to dramatically increase secretion of both factors, resulting in a macrophage phenotype that is not quite M1 nor M2, as high levels of both pro- and anti-inflammatory factors are produced. Addition of dexamethasone, an anti-inflammatory glucocorticoid, resulted in intermediate levels of both factors. Current studies are investigating levels of reactive oxygen species generated in cells exposed to PolyHb and Hp, as well as CD163 expression. PolyHb/MSC interactions will also be studied in the near future, particularly the effect of PolyHb on prostaglandin E2 (PGE2) secretion from MSCs. In Aim 2, PolyHb and MSCs will be encapsulated in alginate sheets, which will serve as wound dressings to localize these components at the wound site and maintain high cell viability. The porous alginate matrix allows for sustained release of PolyHb and MSC-generated factors directly into the wound healing environment. We will develop a computational model to measure diffusivity of PolyHb/MSC factors from alginate hydrogels using an incompressible Navier-Stokes, diffusion-convection phase model in COMSOL Multiphysics software. The diffusivity component will be altered to represent several variables such as starting concentration, alginate porosity, molecular weight, etc. These parameters will be determined based on values found in the literature and will also be fitted to preliminary in vitro measurements. In Aim 3, we have performed in vivo wound healing studies in diabetic mice. These studies demonstrated that PolyHb/MSC dual combination therapy may have a synergistic effect, as this group achieved wound closure at the earliest time point. A second study investigated the healing rate and histological differences between wounds receiving non-polymerized Hb, T-state PolyHb (polymerized in de-oxygenated state) and R-state PolyHb (polymerized in oxygenated state). Complete histological analysis is underway, however, the main difference observed so far is thicker dermal/epidermal layers in PolyHb groups and significantly higher CD31 expression in the T-state group, suggesting more advanced angiogenesis and healing. Next steps will be to complete in vitro tests to further investigate the main in vivo observations, focusing on the PolyHb/MSC interaction and the effect of PolyHb on endothelial cells. Future in vivo studies will monitor macrophage markers in response to the PolyHb/MSC combination therapy.

This work focuses mainly on targeting inflammation in chronic wounds, which is a key barrier to healing. By promoting the M2 anti-inflammatory phenotype in resident macrophages, the wound will be primed for self repair. This research encompasses modeling, in vitro and in vivo studies in order to create an optimal delivery system that targets macrophages in diabetic wounds. The outcome of this work will be an effective, cell- and protein-based chronic wound therapy, along with a deeper understanding of the effects of each component on macrophage phenotype.
PRESENTATIONS


AWARDS

Rutgers Graduate Assistance in Areas of National Need (GAANN) 2015-2017

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THOMAS LINZ
Advisor: Stavroula Sofou

Triple negative breast cancers continue to have poor prognosis due to high proliferation and reoccurrence outside the breast; combined with lack of effective (receptor–mediated) tumor targeting therapeutic modalities. BRCA-1 defective tumors are frequently characterized as TNBC and exhibit aberrant DNA double-strand break repair mechanisms. Our goal is to design a new class of highly effective treatments for TNBC that have the BRCA-1 genetic mutation, using a new class of lipid drug carriers loaded with the highly potent α particle emitter, Actinium-225. Lipid based nanoparticle drug carriers have the potential to make dramatic improvements in the safety and efficacy of a variety of chemotherapeutic treatments for breast cancers.

Specifically, a novel class of lipid nanoparticles with pH triggered receptor binding functionality can greatly increase the selectivity of chemotherapeutic agents. They are designed so that their surface phase-separates into a “sticky patch” when close to a tumor mass, thereby only dosing cells in and around the tumor mass.

Using these targeted nanoparticles loaded with the alpha particle generator Actinium-225 (Ac-225), which has been shown to be highly effective in causing double strand DNA breaks in cells, thereby inducing apoptosis. We aim to selectively dose and kill TNBC BRCA-1 cells, while leaving normal cells undamaged and healthy.

PRESENTATIONS


AWARDS

NJCCR pre-doctoral Fellowship 2016-2018
Cases of advanced solid cancer as of yet have no cures. A challenge for the medical research community is the development of diagnostic and treatment protocols that significantly extend life expectancy and improve quality of life of these cancer patients. Based on the difficulties encountered in treatment, future effective interventions will likely require a combination of therapies to provide effective tumor control, of which antivascular therapy may be a key element. The rationale behind this type of therapy is the observation that solid tumors must develop new vasculature to grow beyond 1-2 mm$^3$ in size. Antivascular therapy aims to kill tumor cells by cutting off blood flow, thereby depriving tumors of the nutrients they need to survive. A number of different approaches have been explored for antivascular therapy, but there are still many challenges to overcome. To be as efficient as possible a therapeutic agent needs to be able to selectively target tumor endothelial cells, preferably using a highly expressed targeted moiety, and be able to avoid becoming entrapped within the delivery vehicle or in an intracellular compartment such as an endosome. The objective of this study is to design a new class of liposomes that can target tumor vasculature, and rapidly and extensively release therapeutic agents intracellularly using the fusion peptide GALA.

The central hypothesis of this study proposes that by targeting Prostate-Specific Membrane Antigen (PSMA) to specifically target the tumor vasculature, and GALA conjugated to specific lipid moieties to act as a pH-sensitive means of fusing drug carrying liposomes with intracellular endosomes, better tumor killing outcomes will be observed. The rationale behind this study is that if a liposome system capable of targeting tumor vasculature and escaping entrapment proves successful, antivascular therapy may become a more viable means of cancer treatment. We are testing our hypothesis through the following specific aims: 1) by determination of the mechanism by which GALA-liposomes induce fusion and release and 2) by demonstrating that PSMA-targeting, GALA-bearing liposomes loaded with doxorubicin exhibit a) selective targeting of tumor endothelium analogs, b) effective release of doxorubicin, and c) enhanced killing of targeted cells.

Thus far it has been established that GALA conjugated to lipid and embedded in a liposome triggers pH-dependent release from vesicles acting as endosome analogs. This is accomplished by GALA interacting with, embedding in, and destabilizing the fluid membrane. It has been shown that the geometric presentation of GALA on the liposome surface has an impact on GALA efficacy. GALA presented in a clustered formation (via a pH responsive membrane) exhibits faster induced release at the average endosomal pH (~5.5) than a uniform formation of GALA. However, at lower pH’s clustered GALA begins to lose efficacy. We believe this is due to enhanced GALA-GALA interaction on the surface of the liposome and are currently investigating this hypothesis. GALA also affects the stability of the membrane it is anchored to at acidic pH, potentially further increasing the liposomes’ ability to release drug within the cell. However, evidence suggests that unlike the fluid membranes mentioned above, GALA is unable to embed in the gel membranes used in this delivery system.

For the in vitro experiments in aim 2, issues have arisen in the efficacy of our targeting ligands and cell models, necessitating delays as solutions and/or alternatives are investigated.

**PRESENTATIONS**


**Locke T**, Sofou S. Membrane-Active PSMA-Targeting Liposomes for Antivascular Chemotherapy, CINJ Annual Retreat, New Brunswick, NJ. May 2016.
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 such as non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular steroid injections 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. Preliminary studies conducted in our lab have shown that the alginate concentration used for cell encapsulation has an impact on the MSC behavior and secretory function. Alginate concentrations of 2.2% (w/v) have been previously used to maintain high MSC viability and induce anti-inflammatory secretory function. Whereas, MSC encapsulated in 1.7% alginate have shown pro-chondrogenic behavior by increasing proteoglycan deposition and increasing transforming growth factor beta (TGF-β) secretion, a common cartilage ECM component and a known chondrogenic factor, respectively. These preliminary studies have led us to hypothesize that by minimally changing the MSC microenvironment, the cells can be tailored for a specific therapeutic function. We aim to ascertain whether intra-articular injection of encapsulated MSC can provide sustained reduction of OA mediated joint inflammation and destruction, and promote re-growth and healing by (1) developing an optimized eMSC therapy that is both anti-inflammatory and chondrogenic and testing its efficacy in (2) an in vitro relevant model of OA and (3) an in vivo chemically induced rodent model of knee OA. Such experiments could provide a powerful new therapy in an otherwise irreversible progressive disease.

To establish an in vitro model of OA, we tested the effect of interleukin (IL)-1 stimulation on primary bovine chondrocytes and conducted a RT-PCR gene analysis of pro-inflammatory cytokine and receptor expression. As expected, IL-1 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. To further assess the chondrocyte responses in the presence of MSCs, chondrocytes were co-cultured with free or eMSCs and gene expression changes were quantified. Downregulation of pro-inflammatory gene expression was observed in all conditions co-cultured with free or eMSC even in the presence of an inflammatory stimulus. This indicates that the use of eMSC as a cell therapy to immunomodulate OA inflammation is a viable option. However, further studies are required to investigate the ability of eMSC to promote anti-inflammatory and chondrogenic function while using other alginate concentrations. Future studies will focus on the eMSC pro-chondrogenic effects on chondrocytes by performing an extracellular matrix (ECM) RT-PCR gene panel and testing for collagen II and GAGs deposition in the co-culture experiments. As well, in order to develop a more comprehensive in vitro model, synoviocytes, the resident cells of the synovial membrane, will be included in a tri-culture system to study MSC-chondrocyte-synoviocyte interactions in OA.

PRESENTATIONS

TBI is a leading cause of death and disability worldwide, commonly resulting from motor vehicle crashes, falls, violence, and sports injuries. It’s estimated 2.6 million emergency department visits, hospitalizations, and deaths each year are the result of TBI across the United States. TBI is most often characterized by a severe physical trauma to the head, followed by a persistent secondary injury cascade. Together, primary and secondary injury result in damage and death of neurons and other neural cells which can cause debilitating loss of cognitive, sensory, and motor functions, for which there currently exists no fully restorative treatment. Cellular damage and death from primary injury occur instantaneous to the injury, but secondary injury persists for weeks and months after the initial insult, offering a potential opportunity for treatment. An obstacle to treatment is the complex, hostile nature of the secondary injury environment, which includes glutamate induced excitotoxicity and oxidative stress from free radicals, which contribute to cell damage and death long after the initial trauma. Delivery of neurotrophic factors to the site of TBI has shown some promise in alleviating these symptoms, particularly with brain derived neurotrophic factor (BDNF). BDNF is critical to neuronal development and survival, but is rapidly cleared in vivo and it is unable to cross the blood-brain barrier at a therapeutically relevant rate, limiting its therapeutic potential. Recently, the investigation of short peptide fragments of the BDNF sequence has yielded a series of peptides that can mimic the activity of the full length growth factor. Our laboratory has routinely created functional biomaterials by covalently immobilizing peptides and small molecules to polymer matrices. The objective of these studies is to develop biomaterials capable of immobilizing BDNF fragment peptides at an injury site for sustained presentation following TBI. To this end, we will investigate (1) the neuroprotective effects of immobilized BDNF fragment peptides against models of secondary injury and (2) a novel means of delivering therapeutic factors for sustained presentation in regions of elevated free-radicals. The central hypothesis of this work is that BDNF fragment peptides covalently immobilized to the site of traumatic brain injury will reduce the effects of secondary injury and improve neuronal survival following TBI. This research will evaluate the neural cell response to BDNF fragment peptides immobilized to both type-I collagen and to poly (ethylene glycol) (PEG) based polymers. The rationale for this research is that sustained presentation of peptides by immobilization to a polymer matrix greatly enhances their efficacy as compared to soluble factors, as shown in previous animal studies by our laboratory. These immobilized peptides may hold a great deal of potential for reducing the effects of secondary injury and improving outcomes following TBI.

PRESENTATIONS


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, Dil 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 NH4Cl to disrupt lysosomal pH, to see if dysfunctional lysosomes 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 mechanism. 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. We have created double mutants of these genes to confirm that they are not additive and act in the same pathway. I have obtained ced-1 GFP reporters that I will use to confirm localization of ced-1 around exophers. If ced-1 localizes around exophers, I will investigate whether this localization is dependent on enpl-1.

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 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.

SARAH MISENKO
Advisor: Sam Bunting

Cancer is caused by the accumulation of mutations affecting genes that regulate cell proliferation and survival. Mammalian cells use two major pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), to repair DNA double strand breaks (DSB) and thus prevent the appearance of mutations leading to cancer. Deficiencies in either of these pathways, for example BRCA1 mutations, can significantly increase predisposition to cancer. The objective of this study is to understand the key contributors to DNA end resection and determine how they influence the choice between HR and NHEJ. Particular genes of interest are Bloom’s helicase (BLM) and Exonuclease 1 (Exo1). Mutations in the BLM gene cause predisposition to tumor development, developmental abnormalities, and chromosomal instability. Exo1 mutations have also been linked to cancer, particularly colorectal and breast cancer. It has been suggested that Exo1 and BLM/Dna2 serve as the two main pathways for long-range resection in yeast, though it has not been shown in mammalian models. A better understanding of the key resection contributors may lead to novel treatment options for BLM and Exo1 mutations or HR-deficient cancers, coined "BRCA-like" cancers. Our central hypothesis of this study is that 1. BLM helicase and Exo1 play important roles in DNA end resection and therefore the commitment to HR and genome integrity and 2. this regulation of double-strand break repair pathway choice is relevant to cancer therapies and outcome. We plan to test this hypothesis through the following specific aims:

1) determine if long-range resection by BLM and Exo1 is required for HR by measuring genomic stability in Blm-/-Exo1-/- double deficient primary mouse B cells
2) determine if deletion of 53BP1 can rescue the "BRCA-ness" of the Blm-/-Exo1-/- cells by measuring resection and HR in Blm-/-Exo1-/-53Bp1-/- primary mouse B cells
3) determine if a DNA-PK inhibitor, Vx984, can be used in cancer treatment as a radiosensitizer by inhibiting NHEJ

Previously, I have shown decreased levels of resection and increased genomic instability in Blm-/-Exo1-/- double-deficient cells compared to WT and the single knockouts. These results suggest that BLM and Exo1
work in redundant pathways for long-range resection. I have also shown that co-deletion of 53BP1, an inhibitor of resection, can rescue the genomic instability seen in the double-deficient cells. In the past year, I have developed a more quantitative approach to measuring resection in primary B cells by measuring the levels of bound RPA, a protein loaded onto resected DNA, via flow cytometry. I have also shown that Vx984, a DNA-PK inhibitor, does inhibit classical NHEJ by measuring the levels of class switch recombination occurring in B cells after treatment with the drug. Using the EJDR reporter cell line for the measurement of HR and mutagenic NHEJ, I showed that Vx984 inhibition of classical NHEJ causes a significant increase in the use of HR and mutagenic NHEJ. I have also performed clonogenic assays and showed that human fibroblasts are more sensitive to irradiation when pretreated with Vx984. These results are being used for pre-clinical studies in connection with Dr. Atif Khan at the Cancer Institute of New Jersey in the hopes of starting clinical trials with Vx984.

AWARDS

Executive Women of New Jersey Graduate Merit Award/Scholarship 2016
NIH Biotechnology Training Program Poster Competition Award 2016
New Jersey Commission on Cancer Research Pre-Doctoral Fellowship 2015 – 2017

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DAN MYERS
Advisor: Patrick Sinko

HIV-1 infection has remained a chronic condition despite current virus-suppressing treatments with antiretrovirals (ARVs) due to a combination of viral latency and the ineffectiveness of drugs in certain anatomical sites. These sites, such as the gastrointestinal (GI) tract, serve as a reservoir for the virus where it can persist in a dormant state in the absence of effective drug doses. This study focuses on developing a platform for delivering anti-HIV drugs, including ARVs, to the GI tract to not only achieve an effective dose, but also sustain that dose in order to eliminate the viral reservoir and destroy HIV infection. Encapsulation in polymeric nanoparticles (NPs) provides a drug delivery vehicle suitable to overcome poor stability in the small intestine and low aqueous solubility. In oral drug delivery research, however, little is known about exploitable mechanisms for NP translocation across the intestinal epithelium. Greater knowledge of potential mechanisms is crucial for achieving effective concentrations of poorly absorbed therapeutics in the intestinal tissue. For this study, NPs are formulated using flash nanoprecipitation (FNP). In order to target the small intestine and improve the permeability of NPs across the intestinal epithelium, an optimal peptide selected by phage display (P19) is conjugated to the NP surface using click chemistry. By incorporating a phage display peptide, we are given the unique opportunity of uncovering a novel mechanism for intestinal uptake. The objective of this project is to use an orally administered peptide-conjugated copolymer NP platform to overcome the poor solubility and low intestinal permeability of anti-HIV drugs in order to deliver effective, sustained doses to the GI tract. We hypothesize that the conjugation of P19 to the surface of drug-loaded block copolymer NPs in an optimized display pattern will result in increased binding and translocation across the intestinal epithelial monolayer and, ultimately, provide a platform to improve the oral bioavailability of anti-HIV drugs. This work is being carried out with the following three specific aims. 1) To engineer polymeric NPs to encapsulate various anti-HIV drugs with sufficient drug loading and sustained drug release profiles. Precise control over NP diameter (from 40nm up to 250nm) has been achieved by altering the block sizes of PEG and PCL in a PCL-PEG block copolymer used for FNP. A quadratic relationship was found between NP diameter and the ratio of PEG repeating units to total repeating units (PEG content) in the PCL-PEG copolymer. This phenomenon has not been previously explored. The minimum NP diameter was reached at 65% PEG content for three different weight ratios of polymer to core material (Vitamin E). Multiple ARVs have been successfully encapsulated by FNP, with drug loadings of up to 32% by weight for darunavir (DRV). Click chemistry reagent pairs were conjugated to the surface of PCL-PEG NPs to effectively crosslink the PEG
corona in order to slow drug release. With a 7% surface density of both methyltetrazine and trans-cyclooctene on PCL-PEG NPs, complete release of DRV in PBS was prolonged from 10 hr to 24hr. Future work for this aim includes improving drug loading and encapsulating novel ARVs including cabotegravir. Sustained release over one week will be achieved by combining NP populations with staggered release profiles. 2) To assess the effect of peptide surface architecture and copy number on in vitro caco-2 cell monolayer translocation and in vivo rat intestinal mucosal uptake of polymeric NPs. A novel peptide sequence (P19) has been identified through T7 phage display. The 12 amino acid peptide sequence has been synthesized, chemically optimized (P19opt), and conjugated to the NP surface through click chemistry. Caco-2 translocation studies have shown a 7-fold increase in NP transport across epithelial monolayers when displaying a low surface density (0.5%) of P19opt. In vivo rat intestinal ligation studies showed that in 3 segments of the intestine (duodenum, jejunum, and colon) NPs displaying 0.5% P19opt had significantly higher translocation into the intestinal mucosa than plain NPs after 2 hr. Alanine scanning (replacing each amino acid with alanine one at a time) of the P19 sequence has shown that each residue is significant for translocation across Caco-2 monlayers. Future studies will further evaluate the effects of both surface density and peptide surface display (branched vs. linear) on NP translocation. Peptides will be resynthesized using D-amino acids to prevent proteolytic degradation in the GI tract. 3) To develop oral formulations using ordered mixtures to reduce NP aggregation in the gut. We hope to utilize a well-established pharmaceutical technique, ordered mixing, to reduce NP aggregation. Ordered mixing has been used to improve the dispersion of poorly soluble drugs, but has not yet been applied to NP suspensions. We will determine proper mixing conditions to form homogenous ordered units of NPs tethered to the surface of large, disintegrant particles. Powdered formulations will be suspended and immediately administered in a ported animal model. The implanted port will allow direct delivery to desired locations in the intestine, and NP uptake will be evaluated.

PRESENTATIONS


AWARDS

PhRMA Foundation Pre-doctoral Fellowship in Pharmaceutics 2015

ANTOINETTE NELSON
Advisor: Patrick Sinko

Both men and women who engage in unprotected receptive anal intercourse (RAI) are at a significantly higher risk of contracting HIV from an infected partner than those who participate in unprotected vaginal intercourse. It is estimated that unprotected RAI results in 10-100 times more incidences per exposure than unprotected vaginal intercourse, exposing a critical need for interventions to prevent viral transmission through this route. Currently, the only FDA approved approach for HIV prevention is the systemic pre-exposure prophylaxis (PrEP) treatment, Truvada. With Truvada, there are toxicity concerns since the entire body is being exposed to antiretrovirals. Patient compliance is also a limitation due to a strict once-a-day oral dosing regimen. A mucosal PrEP would address these concerns by lowering the necessary dosage and frequency while avoiding systemic exposure to drugs. However, a major obstacle to drug delivery in the colon is penetration of the protective epithelial cell barrier and the mucus lining. Through the conjugation of selected cell penetrating peptides to nano-sized delivery systems we have been able to increase and sustain carrier penetration into intestinal tissue. The objective of this study is to develop a foam-based mucosal PrEP
capable of delivering therapeutic agents to the gut mucosal region with limited leakage, good colorectal coverage, and sustained drug release within the colorectal tissue. The central hypothesis is that our mucosal PrEP will maximize surface coverage within the colon, sustain local drug concentrations to minimize administration frequency, provide limited systemic exposure to prevent toxicity, and have very low, if any, anal leakage. Our rationale is that by ensuring minimal leakage of our foam formulation along with sustained drug release, we will increase patient adherence and produce a more effective PrEP therapy for the prevention of HIV transmission. Our study is guided by the following aims: 1) to engineer and evaluate a number of drug-loaded nanocarriers capable of translocating across the colorectal mucus to locally deliver anti-HIV therapies in a sustained manner; 2) to establish target mucosal tissue drug concentrations and correlate drug elimination and release properties from nanocarriers; and 3) to formulate and assess pharmaceutical foams that cover the mucosal surface, leave minimal residual volume upon breaking, and homogeneously distribute nanocarriers throughout the distal colon and rectum. Currently, we are testing a number of foam formulations in vitro and in vivo to investigate foam expansion, breakability and toxicity. We are also enhancing the delivery of nanocarriers to colorectal cells through the conjugation of cell-penetrating peptide bac-7. In vitro and in vivo work for bac-7 optimization is being completed and a manuscript has already been submitted for this work using model PEG conjugates. Another manuscript is currently under preparation for our work using larger bac7-labeled nanoparticles. We are also exploring novel peptides that our lab has identified to increase intestinal uptake. Finally, preliminary pharmacokinetic studies are currently being performed to test key anti-HIV drugs.

In addition to my thesis research, I was a 2014-2016 Fellow in the Rutgers Predoctoral Leadership Development Institute. I am also the current Vice President of the Rutgers Council of Black Graduates and Co-President of the Rutgers Science Policy Group. I have also been involved with numerous STEM outreach programs where I serve as a mentor. These programs include Research in Science and Engineering (RiSE), Leadership Alliance and the Academy at Rutgers for Girls in Engineering and Technology (TARGET). In addition to the papers mentioned above, I have also edited the Solubility and Distribution Phenomena chapter in the Martin’s Physical Pharmacy and Pharmaceutical Sciences, and published three abstracts regarding the described work in the FASEB Journal. I am also now a fellow with the American Foundation for Pharmaceutical Education.

PRESENTATIONS


AWARDS

American Foundation for Pharmaceutical Education Fellowship 2016
Dolores C. Shockley Best Presentation (3rd Place) 2016
ASPET Travel Award 2016
FASEB/MARC Travel Award, Experimental Biology 2016
Recent advances in the field of immunotherapy have resulted in prolonged survival of patients with various types of cancer. Such clinical successes have elucidated the fact that cancer development is not solely dependent on the accumulation of mutations in cells but is also mediated by the ability of the cancer to evade detection and elimination by the immune system. The inability of host immunity to stop cancer development and growth may be due to concomitant challenges faced by the immune system. Epidemiological studies have reported that cancer patients infected with non-oncogenic viruses exhibit an elevated rate of cancer-related death as compared to patients without concomitant viral infections. My lab has developed a model to study this phenomenon. In our model, mice with cancer (melanoma, breast cancer) exhibit faster tumor growth when concomitantly challenged with influenza (or other pathogens) than when such mice are uninfected (Kohlhapp, et al. 2016, Cell Reports, in press). We have discovered that in influenza-infected mice, anti-tumor killer (CD8+) T cells (important for the immune response against cancer) are decreased in the tumor microenvironment (where they are needed to control and eliminate the tumor) and found at increased levels in the lung (the site of influenza infection). These data suggest that infection leads to the shunting of anti-tumor killer T cells from the tumor site to the site of infection. In my rotation, I conducted experiments showing that CD8+ T cell shunting is not solely a function of having two concomitant immunological insults; mice infected simultaneously with influenza and vaccinia did not exhibit any differences in CD8+ T cell migration when compared to mice infected with influenza alone. This finding indicates that anti-tumor CD8+ T cell shunting to a secondary site of inflammation is an immunological phenomenon that may be unique to situations in which the immune system is challenged by a tumor and a pathogen simultaneously. The mechanisms by which anti-tumor CD8+ T cells leave the tumor site and accumulate in the infection site remain unknown; learning about these mechanisms constitutes the core of my thesis project. We hypothesize that shunting of such cells is due to infection-induced changes in chemokines (proteins that mediate the migration of immune cells) within the lung, blood, and tumor microenvironment and due to infection-induced changes in chemokine receptors on immune cells in the tumor. Therefore, I am working to determine the chemokine and chemokine receptor changes induced by infection (via the use of Nanostring mRNA array technology and flow cytometric interrogation of proteins).

PRESENTATIONS


The ubiquitin proteasome pathway (UPP) is the primary mechanism for removal of cellular proteins and is conserved from yeast to humans [1]. Proteins that are targeted for degradation are covalently linked to a small protein called ubiquitin and subsequently degraded by the 26S proteasome [1, 2]. The functions of many players in this pathway are well characterized, although key regulatory aspects of this mechanism remain to be elucidated. For instance, the idea that nuclear proteins are degraded inside the nucleus is not supported by strong experimental data, and in contrast there is compelling evidence that some nuclear proteins are degraded following their export.

The Madura group made a number of discoveries regarding the site of protein turnover. First, we reported that the degradation of some nuclear proteins required export from the nucleus [3]. Second, nuclear substrates that were stabilized in export mutants accumulated inside the nucleus [3]. Third, we also reported
that purified nuclear proteasomes lack peptidase activity unlike cytosolic proteasomes, which are fully functional [4]. Fourth, we reported that Rad23, was identified as a shuttle factor that translocates ubiquitinylated proteins to the proteasome [3]. Fifth, we reported that Sts1 can target proteasomes to the nucleus [3]. Sixth, preliminary data show that Rad23 can be trapped in either the nucleus or cytosol in specific mutants. Collectively, these studies suggest that some nuclear proteins are exported to cytosolic proteasomes that appear to be tethered to the nuclear surface.

The current focus of the Madura lab is to investigate the hypothesis that a nuclear-cytoplasmic transport mechanism is required to transport nuclear substrates to cytosolic proteasomes. I will characterize the role of Rad23 and other shuttle factors in translocating nuclear polyubiquitinylated proteins to cytosolic proteasomes at the nuclear surface. To test this hypothesis I will employ both genetic and molecular approaches. First, I will examine Rad23 function in yeast mutants where its localization is altered. Second, I will identify nuclear targeting motifs through targeted and random mutagenesis to restrict the nucleo-cytoplasmic movement of Rad23. Third, I will functionally characterize mutants, and investigate their role in proteasome-mediated turnover of nuclear proteins and nucleotide excision repair.

Using the temperature sensitive mutants sts1-1 and rna1-1, I found that GFP-Rad23p localizes exclusively to the nucleus in sts1-2, while in rna1-1 it appears to be completely cytosolic. Interestingly the interaction of Rad23 with proteasomal substrates is altered as well. In rna1-1, the interaction of Rad23 with polyubiquitinylated substrates is reduced and in sts1-2, it appears to be increased. This altered substrate interaction is also true for Ddi1, another shuttle factor. In both mutants we can observe a strong stabilization of nuclear proteins, while cytosolic proteins appear to be turned over much more rapidly.

In order to identify possible nuclear targeting motifs, I will use site-directed mutagenesis of specific lysine residues within Rad23p that are suggested by databases that predict nuclear localization sequences (NLS) in proteins. Interestingly, all lysine residues of Rad23p are found within the N-terminal UBL domain. I have already designed and received the oligos and I have prepared the vector for cloning.

In order to identify possible nuclear export sequences (NES), I will be generating a library of mutant Rad23 using random mutagenesis. These mutants will be tagged at the C terminus with GFP to help determine their location within the cell. Moreover, I developed a screen using a yeast strain that contains a double deletion for Rad23p and Rpn10p. The lack of suppression of the severe cold sensitivity in this strain will allow me to select potential mutants. The lack of a GFP signal will additionally help isolate truncated mutants from full length. I have generated the Rad23-GFPp and confirmed its functionality. I optimized the mutagenesis PCR reaction, but experienced problems with the vector. After a single restriction digest the vector efficiently re-ligated when transformed into yeast. I tried several approaches to prevent re-ligation without success, and concluded that my proposed approach will not work. A new strategy is to purchase the service from a company that will generate a library of Rad23 mutants in E. coli. However, this required me to re-clone Rad23-GFP construct adding two restriction sites.

AWARDS

NSF fellowship 2015-2018

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ANTON OMELCHENKO
Advisor: Bonnie Firestein

Traumatic Brain Injury (TBI) is one of the leading causes of death and disability in the United States, and there is currently no successful pharmacological treatment for TBI. TBI is primarily characterized by two mechanisms of injury, primary and secondary injury. Primary injury refers to the mechanical insult to the
brain, which directly damages cellular structures and occurs immediately post-injury. Secondary injury occurs as a consequence of the cellular processes and biological events initiated after the primary insult and takes place over the hours and days following injury. Mitochondrial dysfunction has been previously implicated in the excitotoxicity and cellular damage that occurs during 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 can lead to 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.

During my first year as a student in the Firestein laboratory, my work has primarily aimed to develop a microfabricated device that can be used to model TBI in vitro and to screen pharmacological treatments for injury in a high-throughput manner. The device is constructed using polydimethylsiloxane (PDMS), a silicon-based polymer, and consists of two compartments connected by microfluidic channels. Two separate hippocampal organotypic rat brain slices are cultured within the two compartments, and the two slices create axonal connections through the microfluidic channels. In order to model TBI, uniaxial strain is applied beneath the axons spanning through the microchannels, and mitochondrial function is examined in the axons of neurons expressing DsRed2-mito, a fluorescent protein targeted to mitochondria, using fluorescence and label-free optical scatter imaging. Over the course of my first year, I first learned to dissect and isolate hippocampi of rat pups to produce organotypic slices and to attach and culture the slices in the devices. I maintained the organotypic slices in culture for up to 5 weeks and examined the growth of their axons into the microfluidic channels. In parallel, I used dissociated neuronal culture expressing DsRed2-mito to examine mitochondrial function using fluorescence and label-free optical scatter imaging. I also treated the neurons with NMDA to chemically model TBI in culture. I examined mitochondrial dynamics using live imaging and found excessive fission as a result of the NMDA application. Additionally, I wrote a macro in ImageJ to analyze various mitochondrial parameters, such as size and roundness, to quantify changes in mitochondrial dynamics in response to injury. My next goal is to subclone DsRed2-mito into a viral vector to transduce organotypic slices and to examine mitochondrial dynamics in organotypic slices after the application of injury in the form of uniaxial strain.

KATE (Fitzgerald) O’NEILL
Advisor: Bonnie Firestein

Traumatic brain injury (TBI) affects millions of people in the U.S. each year, and unfortunately, there is still no agreed upon treatment that can effectively limit the progression of TBI, perhaps due to the two-fold nature of the injury. The primary physical injury leads to a secondary chemical cascade, particularly glutamate-induced excitotoxicity, that is toxic to cells. Promising drugs have attempted to mitigate toxicity by blocking the N-methyl-D-aspartate (NMDA) glutamate receptor, but they have failed therapeutically because low amounts of glutamate are still necessary for neuronal function. Instead, this dissertation uses brain-derived neurotrophic factor (BDNF), a prevalent central nervous system growth factor, to activate neuronal survival pathways. If treatment with BDNF can overcome the negative effects of glutamate-induced excitotoxicity, this will not only improve therapies for brain injury victims but also for nearly all patients with neurodegenerative diseases, which share excess glutamate as a common injury mechanism with TBI. Moreover, in the case of TBI, glutamate-induced excitotoxicity can be more harmful than the initial physical injury because it causes widespread damage by injuring healthy tissue outside the trauma site. Finding a solution for this aspect of TBI is of utmost importance for improving treatment for TBI patients.

Thus, the goal of this dissertation is to determine whether BDNF can help neurons recover from the chemical effects of brain injury, specifically glutamate-induced excitotoxicity. My hypothesis is driven by the fact that calcium influx through synaptic NMDA receptors (NMDARs) promotes neuronal survival by triggering cAMP
response binding element (CREB) and BDNF expression, whereas calcium influx through extrasynaptic NMDARs results in cell death, which occurs during glutamate-induced excitotoxicity. I am testing my hypothesis by examining changes at the network level to better understand the injury and recovery processes. Previous work has determined how BDNF affects the structure and function of neurons, but these studies have primarily focused on single cells. By incorporating network-level experiments, I am showing how changes in individual neurons affect the overall network. To monitor the activity of neuronal networks, I have employed microelectrode array devices (MEAs), which have been used by our laboratory and others to understand how networks develop and recover from injury.

The use of MEAs in my work has allowed me to determine that BDNF significantly alters network synchronization without altering the amount of overall or organized activity. Additionally, I have found that hippocampal neurons are significantly more sensitive to injury by excess glutamate than are cortical neurons. I have also developed an automated analysis program to more accurately detect the network activity of hippocampal neurons, which are more variable in their activity than are cortical neurons. Currently, I am evaluating whether BDNF can be neuroprotective against excitotoxic damage to hippocampal neurons. My preliminary data suggest that BDNF may be neuroprotective at 24 hours after excitotoxic injury but not at 72 hours after injury, indicating that there exists an optimal time window for treatment with BDNF.

PRESENTATIONS


AWARDS

U.S. Department of Education GAANN Fellow 2016 - 2017
SWE Region E Scholarship 2016
Honorable Mention, Ford Foundation Dissertation Fellowship 2016
SfN Trainee Professional Development Award 2016

Mutations in BRCA1 are responsible for approximately 5% of cases of breast cancer, and there are few treatment options that substantially alter the probability of disease onset in individuals with BRCA1 mutations. Deletion of the DNA damage response factor, 53BP1, significantly reduces the rate of tumor formation in mice carrying BRCA1 mutations, by a mechanism that appears to depend on restoring the activity of the homologous recombination pathway (HR) for repair of DNA double-strand breaks (DSB). 53BP1 has been shown to act as part of a complex involving RIF1 and PTIP. Data generated thus far indicate that BLM, a DNA helicase, is a novel regulator of genomic instability in BRCA1-deficient cells and may act as a downstream effector of 53BP1 via association with RIF1. Our long-term goal is to elucidate the molecular mechanism of DNA resection during HR-mediated DNA repair, which may enable the development of novel treatment options for cancers that arise from genomic instability, particularly BRCA1 mutations. The objective of this study is to elucidate the function of BLM helicase in DNA resection. The central hypothesis of the proposed experiments is that BLM regulates DNA DSB repair through regulation of HR. I plan to address my hypothesis through the following Specific Aims:
1. Identify genetic relationship between BLM and other DNA repair factors (BRCA1 and 53BP1) that modulate DSB resection and genomic instability. Our working hypothesis is that the capacity of BLM to mediate DSB resection is impacted by 53BP1, possibly through a downstream effector, RIF1.

2. Identify molecular interactions between RIF1, 53BP1 and BLM that are responsible for maintaining genomic stability. Our working hypothesis is that BLM is a downstream effector of 53BP1 via association with RIF1.

3. Determine if BLM inhibits synapsis during spermatogenesis. Our working hypothesis is that conditional deletion of BLM in germ-line cells will not block repair of meiotic DSBs in BRCA1- and 53BP1-deficient spermatocytes and will result in proper spermatogenesis.

Thus far, I have obtained evidence that suggests that BLM impacts homologous recombination and mediates genomic instability in BRCA1-deficient cells. Over the past year, I have acquired additional data to establish the role of BLM in mammalian DSB repair. The deletion of BLM is able to rescue genomic instability and HR in mice with the hypomorphic BRCA1Δ11/Δ11 allele, but not the RING domain deficient BRCA1Δ2/Δ2 allele. This suggests that the rescue of HR in BRCA1Δ11/Δ11 BLM Δ/Δ double deficient cells is specific to the BRCA1Δ11/Δ11 hypomorphic allele and may require a functional RING domain. Currently, I am testing whether the helicase activity of BLM is required for the rescue of HR in BRCA1Δ11/Δ11 BLM Δ/Δ cells. Furthermore, I am testing whether deletion of other BLM interacting proteins, TOPIIa or RMI1, or RecQL5 helicase is able to rescue the HR deficiency phenotypes in BRCA1Δ11/Δ11 cells.

To elucidate the interaction between BLM and RIF1, I have generated and expressed mutant constructs of BLM in human cell lines. Through co-immunoprecipitation experiments using various deletion constructs of BLM, I have identified that RIF1 interacts with BLM at the N-terminal region of BLM. This finding demonstrates that the region of BLM that interacts with RIF1 is the same region that is known to interact with RMI1, suggesting a mechanism of regulation of BLM function via its interacting partners.

In addition, I have generated conditional deletion of BLM in germ line cells by breeding Vasa-Cre mice with BLMf/f mice to identify the role of BLM in meiotic crossover during spermatogenesis. Through histological analysis of testis cross-sections from these conditional deletion mice, I have determined that deletion of BLM does not rescue the meiotic defect present in BRCA1-/- 53BP1-/- null male mice. This suggests that the meiotic defect in BRCA1-/- 53BP1-/- null male mice may not only be due to a failure in crossover formation, as deletion of BLM, a pro-recombination factor, does not rescue the meiotic defect.

PRESENTATIONS


AWARDS

New Jersey Commission on Cancer Research Pre-Doctoral Fellowship 2015-2017

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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 inability for self-repair. Current surgical interventions include the practice of microfracture and autologous chondrocyte 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 also 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. Some current models have utilized mechanical preconditioning of the cell-seeded scaffold 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 can condition chondrocytes to produce the desired ECM while in vivo, thereby obviating the time and expense of preconditioning in vitro as well as the risks of using growth factors. The objective of my study is to develop a polymer fiber-reinforced composite scaffold that can be seeded with autologous chondrocytes and immediately be 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 influenced 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 of the project 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 vivo, and (3) test how an expandable scaffolds can integrate into the surrounding native cartilage tissue when laterally compressed prior to insertion. Our lab has developed a prototype scaffold consisting of woven and sintered polycaprolactone fibers with an alginate-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 alginate. We are currently developing a substrate with proteoglycan components, such as hyaluronic acid, to improve the viscoelasticity as well as promote chondrocyte function. Once the scaffold has the desired mechanical properties, we will test its ability to support an autologous chondrocyte population in vivo in a rabbit 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


Christopher Rathnam
Advisor: Ki-Bum Lee

Regenerative medicine is a continually growing field that has attempted to revolutionize the healthcare industry. 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. Nevertheless the need to control cellular fate is important for the potential therapeutic use of stem cells, especially autologous stem cells. There is therefore a need to develop a non-viral and non-integrative method to alter gene expression both in vitro and in vivo. 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.

During my first year in graduate school I have focused on improving this NanoScript platform with the goal of making it more clinically relevant. I have been developing a novel activation domain that can increase the efficiency of NanoScript as well as a light inducible dimer system to make NanoScript stimuli responsive. I have learned nanoparticle synthesis, as well as conjugation chemistry for biomolecules on the surface of NanoScript and have performed qRT-PCR to quantify gene expression of NanoScript when delivered to cells. In addition I have studied multiple DNA-binding domains that can be potentially adapted for NanoScript with the goal of improving the selectivity and specificity of the NanoScript platform to minimize off target binding. This year I am focusing on the use of novel hybrid nanomaterials for controlling cell behavior to work synergistically with NanoScript. I have used a variety of techniques including FE-SEM, TEM, DLS, and Zeta potential to characterize NanoScript and have performed MALDI-ToF to characterize the moieties that will be bound to NanoScript. I hope to optimize the platform to generate IPSC derived neurons for the treatment of TBI, SCI, and neurodegenerative diseases.

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**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. The screen was successfully enriched for known genes involved in PEV, as well as identified genes with no previously known role in heterochromatin formation and maintenance.

Surprisingly, integration density profiling of the mutagenized genes enriched in the screen after several rounds of selection for loss of pericentromeric heterochromatin silencing also produced a number of essential genes involved in DNA replication. 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 address this, we are developing a method to couple isolation of actively replicating regions with proteomic analysis of specific genomic loci to determine whether the components of the replication fork and associated proteins differ between heterochromatic and euchromatic genomic regions. Using this method, we aim to gain insight into the mechanism by which heterochromatin inheritance is coordinated with DNA replication to ensure that heterochromatin is maintained throughout the cell cycle and transmitted to subsequent generations with a high degree of fidelity.
Schizophrenia is a devastating mental illness that significantly reduces quality of life and is characterized by positive symptoms (such as delusions and hallucinations), negative symptoms (isolation, withdrawal, and self-neglect), and cognitive deficits. The etiology and mechanisms behind this disorder are poorly understood, and effective treatments for schizophrenia represent a critical, unmet medical need. In order to develop more effective pharmaceutical therapies, it is necessary to further elucidate the cellular and molecular mechanisms underlying the development of this disorder. One of the hallmark cellular features of individuals with schizophrenia is a decrease in dendrite branching, which can lead to impaired neuronal connectivity and plasticity. Nitric Oxide Synthase 1 Adaptor protein (NOS1AP) is a protein encoded by a schizophrenia susceptibility gene and is elevated in the dorsolateral prefrontal cortex in individuals with schizophrenia. Interestingly, NOS1AP reduces dendrite branching in embryonic rat hippocampal neurons when overexpressed, suggesting that NOS1AP may play a key role in producing the dendritic branching abnormalities in the disease. Additionally, NOS1AP is implicated in NMDA receptor (NMDAR) hypofunction, which is thought to be a major contributor to the positive, negative, and cognitive symptoms of the disorder. These data suggest that NOS1AP may serve a viable drug target for developing improved treatments.

The overarching goal of my project is to determine how commonly prescribed drugs and NMDAR agonists affect NOS1AP expression and dendrite branching, using both in vitro and in vivo models. If either of these classes of drugs reduces expression and/or correct dendrite branching abnormalities that occur with NOS1AP overexpression, this would suggest that they might be an effective treatment option. To accomplish the first objective, I treated embryonic rat cortical neurons with antipsychotics or NMDAR agonists. Interestingly, Western blot analysis revealed that only the NMDAR agonists significantly reduced expression of NOS1AP while the antipsychotics had no effect. Next, we investigated the effects of NMDAR agonists on dendrite branching when NOS1AP is overexpressed. To do this, I transfected cultured rat cortical neurons to overexpress NOS1AP, treated the neurons for 24 hours with NMDAR agonists, and then fixed, immunostained, and performed Sholl analysis to examine dendrite branching. I found that the NMDAR agonists partially restored dendrite branching to normal levels when NOS1AP was overexpressed, suggesting that they mediate a positive effect on the reduced dendrite branching seen in schizophrenia. However, we are currently testing longer treatment times to determine whether the effect increases over time. Additionally, we are now extending this work to an in vivo model system. Specifically, we are injecting rats with antipsychotics and NMDAR agonists to determine if NMDAR agonists reduce NOS1AP expression in vivo, as we found in our in vitro study. This evidence would further suggest that NMDAR agonists are potential treatment options for patients with schizophrenia. Lastly, to incorporate a direct human application to this research, we are collaborating with Dr. Steven Silverstein at the Rutgers University Behavioral Care to examine possible expression of NOS1AP in human cheek cells and whether expression is elevated in cheek cells of patients with schizophrenia, as it is in the brain. If we detect elevated levels in cheek cells of patients with schizophrenia, our data would provide the first biomarker for schizophrenia. Because NOS1AP has never been studied before in cheek cells, we first determined whether NOS1AP is detected in these cells in healthy individuals. Western blot analysis revealed consistent expression of NOS1AP in all subjects. We are now repeating this study in patients with schizophrenia to determine if they have elevated NOS1AP expression compared to healthy controls. In sum, we are using several model systems to not only unravel NOS1AP action and its role in schizophrenia but also to investigate NOS1AP as a possible biomarker and diagnostic tool for this devastating disorder.

PRESENTATIONS

Svane K. Effects of Antipsychotics and NMDAR Agonists on Dendrite Branching and NOS1AP Expression in Schizophrenia, Neuroconnections Club, July 2016

Traumatic brain injury (TBI) affects approximately 1.7 billion people each year and is the leading cause of death in people under 45 years of age in the United States. TBI is primarily caused by deformation of brain tissue due to mechanical trauma, followed by rapid release of glutamate and subsequent excitotoxicity (secondary damage). Additionally, the early phase of secondary injury causes loss of dendritic spines and formation of varicosities along dendrites and axons. Several groups have reported spine loss correlating with behavioral deficits following brain injury, and further evidence shows that aiding neurons in spine reemergence drastically improves functional recovery. Therefore, understanding the signaling pathways involved in spine maintenance and reemergence may lead to additional targets for pharmacological intervention in TBI.

One pathway that undergoes a dramatic increase in signaling activity after injury is the PI3K/Akt/mTORC1 pathway and it has become a focus for drug development for the treatment of central nervous system injuries. The PI3K/Akt/mTOR pathway plays an essential role in cellular growth and repair of neurons and is susceptible to modulation through glutamate receptor-mediated signaling. Other groups have found that excitatory levels of amino acids stimulate pathway activity, while excitotoxic levels of amino acids lead to pathway suppression. While many studies have focused on teasing out the role of the PI3K/Akt/mTOR pathway in damage and repair, the results of these experiments have yielded conflicting results on whether activation of PI3K/Akt/mTOR signaling is beneficial or detrimental for neuronal and functional recovery.

Additionally, our laboratory published evidence showing that cytosolic PSD-95 interactor (cypin), a guanine deaminase, regulates dendritic spine reemergence and promotes neuronal survival in an in vitro glutamate induced injury model of TBI. Our unpublished data show that cypin also interacts with the proteasome complex, which plays a critical role in formation of long-term memories and of which activity is disturbed after TBI. While viral overexpression of cypin in vitro increases frequency of miniature excitatory postsynaptic currents (mEPSC) in rat hippocampal neurons, knock down of cypin increases the number of dendritic spines in vitro and improves cognitive scores in vivo. Understanding the role cypin may play in TBI could be crucial for increasing recovery post TBI.

Although TBI has been studied for many years, and its effects on learning and memory are well known, the molecular mechanisms of how TBI affects these cognitive functions are still largely unknown. Therefore, the broad objectives of this study are to 1) evaluate activity of the PI3K/Akt/mTOR pathway and its downstream targets following neuronal injury in vitro and 2) investigate changes to cypin expression and function after injury in vitro and in vivo.

The specific aims are to 1) investigate electrophysiological properties and activity of neurons following manipulation of Akt and several downstream targets, such as GSK3β, FOXO1, and mTORC1, prior to sublethal NMDA-induced injury; 2) investigate changes to cypin protein expression in cortex and hippocampus of mice subjected to controlled cortical impact (CCI); 3) determine the effect of pharmacological modulation of cypin on recovery of electrophysiology, morphology, and cognitive ability in mice after CCI; 4) determine the effect of manipulation of the proteasome activity and cypin on recovery of electrophysiology and morphology after injury in vitro and cognitive ability following injury in vivo; and 5) assess the role of cypin and proteasome on recovery of cognitive function in mice following lateral fluid percussion (LFP) injury.

To date, our analysis shows that exposure of neurons in vitro to high levels of NMDA results in phosphorylation of Akt, S6, and GSK3β at two, but not 24, hours following injury. Phosphorylation of these targets has been associated with pro-survival pathways; however, electrophysiological recordings show that NMDA-induced injury causes a significant decrease in spontaneous excitatory postsynaptic currents at both two and 24 hours post-injury. This phenotype can be reversed by inhibiting mTORC1 and phosphorylation of GSK3β. Additionally, inhibition of mTORC1 and suppression of GSK3β activity decreases the number of
neurons with varicosities following NMDA-induced injury. NMDA appears to act independently of Akt to disrupt neuronal morphology and electrophysiology since inhibition of Akt does not reverse the effects of NMDA. Together, these data demonstrate the Akt-independent importance of mTORC1 and GSK3β in maintaining proper neuronal electrophysiology following injury.

Analysis of brain tissue collected from animals subjected to mild CCI revealed increased cypin protein expression in the cortex at 1 and 7 days and in the hippocampus at 7 days post-injury. We predict that this induction in expression leads to 1) neuronal hyperactivity, as supported by our in vitro electrophysiology data, that disrupts activity of neuronal circuits and may cause epilepsy and 2) inhibition of the proteasome complex, effectively leading to deficits in long-term potentiation and cognitive ability.

Our in vitro data show that pharmacological manipulation of the enzymatic activity of cypin leads to recovery of neuronal electrophysiology following NMDA-induced injury. Preliminary data generated by our collaborator, Dr. David Meaney, show that activation of the enzymatic activity of cypin improves scores in accelerated Morris Water Maze, suggesting improved cognitive ability. We are currently expanding upon this potentially therapeutic approach and are testing small molecule probes to activate or inhibit the activity of cypin in mice subjected to CCI.

Finally, to investigate the effects of manipulation of cypin and proteasome activity on recovery after brain trauma, we will virally induce cypin knockdown or overexpression in mice and pharmacologically modulate the activity of the proteasome complex, and we will assess the effect of these manipulations on electrophysiology and morphology of neurons in vitro and cognitive recovery in vivo.

These studies will give additional insight into the role of the PI3K/Akt/mTOR pathway and cypin in the healthy and injured brain and aid in identification of new therapeutic approaches for use in humans.

AWARDS
Joint NJCSCR Spinal Cord Injury Techniques Training Travel Grant 2016
NJCBIR Pre-Doctoral Fellowship 2016-present
NSF I-Corps Entrepreneurial Co-Lead (PI: Federico Sesti) 2016-present

CORINA WHITE
Advisor: Ronke Olabisi

The retina is a complex system composed of several highly specific cell types that are able to react to light and produce a signal transmitted to the brain, which becomes vision. Retinal dysfunction through the death of non-proliferating cells is implicated in several disorders that lead to loss of vision, including age related macular degeneration (AMD), the leading cause of blindness in developed countries. In AMD, the retinal pigment epithelial (RPE) cells, a monolayer that supports the neural retina, becomes dysfunctional, leading to a change in the properties of the blood retinal barrier of which the RPE comprises. The changed properties of the blood retinal barrier include decreased hydroconductivity and ability to transport nutrients to the neural retinal. This leads to eventual neural retina death. Previous studies, including a phase II clinical trial, show great promise for cell-based therapies in the treatment of retinal degenerative diseases. However, there are limitations of these therapies, including cell death and the inability of the injected cells to form the proper architecture. Therefore, a major area of current research is the development of a scaffold to support RPE transplantation and long-term viability of functional RPE cells. Previous research in this area has shown that implanted scaffolds lead to an inflammatory response and de-differentiation of the implanted RPE cells. In additions to these problems, prior scaffolds have not undergone mechanical property characterization, and limited work has been done to design a chemically modified scaffold to improve the outcome. My research
seeks to understand how mechanical and chemical scaffold cues can be used to drive successful RPE transplantation. The central hypothesis of this research is the poor results of RPE transplantation in vivo are due to current scaffold properties not matching the native retinal environment, and that key scaffold cues can be used to enhance RPE cell viability and function.

Our research efforts have been focused in two main arenas: (1) mechanical characterization of scaffolds and cell studies on scaffolds of varying moduli; and (2) chemical stimulation of cells with PEGylated growth factors and signaling molecules. Results have demonstrated that the elastic modulus of the scaffold affects cell adhesion, viability, and metabolic activity, with the highest activity seen in scaffolds that mimic the native retinal modulus, and gene expression. We have also demonstrated that changes in elastic moduli alone can elicit varied expression of inflammatory markers in RPE cells. The focus of future studies will seek to understand how chemical functionalization of scaffolds with Activin A, a signaling molecule shown to promote the mature RPE phenotype, impacts cell viability and function. We anticipate that these design parameters, mechanical properties and chemical functionalization, will positively regulate cell metabolism and protein expression, in order to overcome the hurdles that current scaffolds face.

PRESENTATIONS

White C, Olabisi RMR. The effects of mechanical and chemical scaffold surface cues on retinal pigment epithelial cells. World Biomaterials Congress, Montreal, Canada 2016


White C, Olabisi RMR. The effects of scaffold mechanical cues on retinal pigment epithelial cells 30th Annual Laboratory for Surface Modification & Institute for Advanced Materials, Devices, and Nanotechnology Symposium, Piscataway, NJ 2016

AWARDS

US Department of Education GAANN Fellow 2016

There are many indications for injecting a treatment directly into the brain, bypassing the restrictive blood brain barrier (BBB). For some of these, such as brain cancer, the drug would be best administered chronically over time, rather than in one large dose. However, to chronically administer a drug directly to the brain would require multiple surgeries, which is disadvantageous and increases risk of infection. The alternatives are either to insert a delayed-release capsule or slowly degrading drug wafer into the brain, or to use a device such as the Ommaya Reservoir, which consists of a refillable reservoir that sits just under the skin of the scalp, and a catheter that extends into the brain. In the latter case, since the reservoir can be refilled, it enables administration of multiple drug types or doses over time without the need for multiple surgeries. However, rapid cerebrospinal fluid (CSF) clearance and slow diffusion into the brain both remain barriers to achieving reasonable drug penetration into the brain. The tortuous geometry in the brain, coupled with proteoglycan obstruction and cell-receptor binding in the interstitial fluid all lead to slow diffusion into the
brain. Furthermore, the CSF is completely replaced about 4 times per day, clearing away chemicals in the brain as it goes, so even if a drug bypasses the BBB by way of an Ommaya device and is able to penetrate far enough, it will still be cleared quickly, before it is able to produce its full effects.

Therefore, we are investigating the use of chemotherapy drugs in the Ommaya device in combination with CSF modulators to either slow down or speed up the rate of CSF production. Depending on the source of the problem, lack of diffusion or excessive clearance, either pushing the drug further by increasing the rate of CSF production (for lack of diffusion) or allowing it more time to penetrate by decreasing the rate (for too-fast clearance), we will enable the drug to penetrate further and have a more potent effect before it is washed away. There are two arms to this project: redesigning (miniaturizing and optimizing) the Ommaya device for use with laboratory rodents, and testing the CSF-modulating drugs, first in vitro, and then in vivo. My work has been split between device design and initial testing of the CSF-modulating compounds.

For the microOmmaya device, I have been learning to use CAD/CAM (computer assisted design/manufacture) software, and the first models we have designed are for catheter optimizations, since we are working on a much smaller scale than the medical device currently in use. I have designed model devices for empirically testing different catheter lengths and the effects of various machining parameters on the catheter portion of the microOmmaya. These models will be tested by evaluating the accuracy of the final product’s dimensions and by investigating pressure-flow relationships. My work with the CSF compounds began with elucidating the IC50 values for acetazolamide, verapamil (CSF modulators), temozolomide and doxorubicin (chemotherapy drugs) in three rat glioblastoma/glioma cell lines, C6-LacZ, RG2 and F98. Once toxicity data for each chemical with each of the cell lines was obtained, I tested each CSF modulator with each chemotherapy drug, looking for a synergistic shift in toxicity, by using proliferation assays (MTS and Glo). The data that I obtained has indicated that the drugs seem to act independently, so if the in vivo experiments show an increased anti-cancer response in rats treated with both CSF modulators and chemotherapy drugs (vs. chemotherapy alone), it will not be due to an interaction between the drugs, and more likely be due to modulation of the CSF flow. In the future, I will be focusing more on optimizing the biocompatibility of the microOmmaya device, and I will also test the drugs in vivo (in brain tumor-bearing rats) using the device.

During my first year, I did a rotation in the laboratory of Dr. Prabhas Moghe, growing human induced pluripotent stem cells (hiPSCs) and inducing them to differentiate into dopaminergic neurons for the purpose of eventual encapsulation and implantation in the brain to reverse some of the symptoms of Parkinson’s Disease, which is characterized by damage to dopaminergic neurons in the brain. I learned how to differentiate hiPSCs using a prescribed set of growth factors and other small molecules and encapsulate them for transplantation. I also observed mouse surgeries and tests for Parkinsonian behavior in mouse models of Parkinson’s disease.
PAPERS PUBLISHED


PAPERS PUBLISHED


# Appendix D: Rutgers University Biotechnology Training Program

## Industrial Rotations

### Summer 2016

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STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Przemyslaw Swiatkowski

Department and Advisor: Cell Biology and Neuroscience, Dr. Bonnie Firestein

Corporation: Fox Chase Chemical Diversity Center, Inc.

Mentor: Dr. Katie Freeman

Project Title: N/A

General Objective of the Project: Analyze novel compounds for antibacterial and antifungal properties.

Student’s Contribution to the Project: I analyzed over 50 compounds for their antibacterial and antifungal properties, selected a small number of potent probes and assessed toxicity on cell lines in cytotox assays. Out of all compounds tested, several were selected for further PK and PD studies in mice.

Techniques Learned: Minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), cytotox assay, ELISA.

Student Comments on the Company and Mentor: FCCDC is a small company that heavily relies on analytical chemistry for novel drug design and discovery. The biology department consists of only one person, Dr Katie Freeman, which allowed for a large amount of hands on experience and exposure. I was also encouraged to attend weekly progress meetings, quarterly company meetings, discuss and propose new ideas to the personnel and leadership at FCCDC.

Additionally, the company is located in the Pennsylvania Biotechnology Center in Doylestown, PA, which is also home to the Baruch S. Blumberg Institute, and many other small biotechnology companies. This allowed me to meet and network with scientists at other companies, which gave further insight into the world of industry research. My overall experience was great and I recommend completing an internship at FCCDC.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Madison Godesky

Department and Advisor: Biomedical Engineering Department, David Shreiber

Corporation: Merck Research Laboratories, Kenilworth, NJ

Mentor: Chakravarthy Narasimhan

Project Title: Syringe Compatibility of High-Concentration MK-xxxx

General Objective of the Project: To evaluate pre-filled syringe candidates for compatibility with a high-concentration formulation of one of Merck’s current mAb drug products. The high-concentration formulation is essential for subcutaneous delivery since the injection volume is limited to <2mL. Subcutaneous drug products are therefore formulated at concentrations >100mg/mL, which contributes to high viscosity drug substances and leads to molecular crowding. These properties cause limitations during manufacturing and administration, as well as aggregate formation and other stability issues. Because drug substances for subcutaneous delivery tend to have high viscosities, administration using a device, such as a pre-filled syringe, is preferred.

Student’s Contribution to the Project:
- Assessed the stability of high-concentration drug substance in pre-filled syringe candidates. Specifically, for each syringe candidate, I evaluated: Drug product concentration, turbidity, particle counts, aggregation, oxidation, and injection force under various conditions of temperature, agitation, and light exposure stresses.
- Determined if silicone and tungsten contained in glass syringes are compatible with the product
- Examined how the syringe material – glass vs. plastic – affects the product stability
- Characterized the shear thinning properties of high concentration drug substance
- Determined how pH and PS-80 influence syringe influence injectability

Techniques Learned: I learned how to design stability studies in downstream biologics development.
- Microflow Imaging (MFI) for particle quantification
- Optical density measurements to evaluate turbidity
- Absorbance measurements to calculate protein concentration stability
- Data interpretation from size exclusion chromatography, hydrophobic interaction chromatography and ion exchange chromatography results
- Syringe injection force measurements using the Mechanical Testing System (Instron)
- Light exposure testing under ICH guidelines
- Rheology using mVROC viscometer
- Formulation of high viscosity, high concentration materials

Student Comments on the Company and Mentor: Working at Merck was a great experience from start to finish. Everyone I worked with was helpful and pleasant and I liked that they trusted my judgment and allowed me to take ownership of the project. I learned a lot at Merck both in and out of the laboratory and am grateful for the experience.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name:  Jeremy Anderson

Department and Advisor:  Biologics & Vaccines – Bioprocess Development – Potency Analytical Testing

Corporation:  Merck & Co., Inc.

Mentor:  Musaddeq Hussain

Project Title:  Development of droplet digital PCR for quantification of host cell residual DNA in biologics

General Objective of the Project:  Quantification of host cell residual DNA in biologics is necessary for ensuring drug purity and safety. Currently, quantitative real-time PCR (qPCR) is the pharmaceutical industry standard for quantification of host cell residual DNA in biologics substance. Droplet digital PCR (ddPCR) is a new method of PCR which partitions the reaction into thousands of nano-liter sized droplets and offers increased sensitivity with lower background. Here we develop and evaluate a ddPCR method for the quantification of host residual DNA in biologics produced in \textit{E. coli} which can offer increased sensitivity and become the new industry standard for the quantification of host residual DNA in biologics.

Student’s Contribution to the Project:  My summer project was to develop a droplet digital PCR (ddPCR) method to quantify host residual DNA in biologics produced in \textit{E. coli}. Once becoming trained using the ddPCR, I tested the linearity and range of the method with \textit{E. coli} genomic DNA. After understanding the range and limit of quantification of the method, I tested the method in five drugs for precision, accuracy, and repeatability.

Techniques Learned:  I learned how to use the droplet digital PCR (ddPCR) machine (BioRad) proficiently during the course of this project. I additionally learned how to program and use automated liquid handlers (TECAN), run fit for use tests using quantitative real-time PCR (qPCR), and run in-process samples for host cell residual protein using ELISA.

Student Comments on the Company and Mentor:  I really enjoyed working for my mentor Musaddeq Hussain and for Merck. My mentor was very helpful and taught me a lot – he facilitated independent thinking and working and provided guidance when necessary, giving me the freedom to learn and grow as a scientist. My group was also amazing – they all were very friendly and taught me many new techniques and instruments, bringing me in as if I were one of their own. I really enjoyed working at Merck, and I learned a lot from other groups, seminars, conferences, and poster sessions offered.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Ryan Guasp

Department and Advisor: Cell and Developmental Biology; Dr. Monica Driscoll

Corporation: Center for Medicine in the Public Interest

Mentor: Dr. Robert Goldberg, Vice President

Project Title: Impact of Longevity on Wellbeing and Society

General Objective of the Project: I worked closely with Dr. Goldberg in studying the implications of new and current biomedical technologies for human healthspan and longevity. The ultimate goal of the project is to create a book which conveys recent biomedical advances in longevity research to the general public, and to make specific governmental policy suggestions.

Student’s Contribution to the Project: I conducted background research for the project and wrote several sections of the initial manuscript dealing specifically with the biology of longevity.

Techniques Learned: Translating primary scientific literature into concise and accurate writing for a general audience.

Student Comments on the Company and Mentor: The Center for Medicine in the Public Interest would be a good fit for anyone interested in American science policy, or regulatory aspects of pharmaceuticals or medical devices. Dr. Goldberg was a pleasure to work with.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Dharm Patel

Department and Advisor: Biochemistry, Samuel F. Bunting

Corporation: Leo Pharma, Inc

Mentor: Elise Costa, Ellen Bortniker

Project Title: Internship in Medical Strategy & Scientific Affairs

General Objective of the Project: Perform a variety of tasks with groups within the department to learn about pre- and post-marketing issues as they relate to Medical Affairs in the dermatology therapeutic area.

Student’s Contribution to the Project: Pharmacovigilance (PV): Researched and presented on best practices regarding social media PV and Biologics PV Reporting; conducted Adverse Event follow-ups; authored Picato® PADER reports for FDA submission

- Medical Information: Established a work-flow for organization of scientific literature pertaining to dermatology within Elsevier’s QUOSA Scientific Literature Management System; Established procedure for mining of newly published articles in Psoriasis and Actinic Keratosis; Prepared material and presented training course for Medical Affairs directors, MSLs, and Med Info team on Scientific Information Management

- Health Economics & Outcomes Research: Compiled competitor clinical data for the Enstilar® Budget Impact Model for dissemination to Payors and Formularies to demonstrate cost effectiveness of product compared to biologics

- Medical Science Liaison Team: Field visits to HCPs with dermatology MSL; identified relationship building and management techniques with KOLs, SLs, HCPs; reviewed FDA guidelines related to MSLs; identified MSL cross-functional interactions within company; attended MSL Society Webinars on KOL engagement and technology use

Techniques Learned: QUOSA Scientific Information Management, FDA PADER report writing, presentation skills, Excel, FDA guidelines (PV, MSL team, Medical Info)

Student Comments on the Company and Mentor: The internship provided great experience in how companies navigate providing scientific support to products post-marketing. Areas that the MSSA team provided support included, pharmacovigilance, medical information, health economics and outcomes research, and having a field based team of pharmacists, and PhDs to act as liaisons between pharma companies and HCPs. We had a weekly mentoring meeting to discuss progress on various projects. I had projects in each of the four divisions, but the workload was doable. Overall, the experience was great and I would recommend it to other students.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Salim Ghodbane

Department and Advisor: Biomedical Engineering- Michael G Dunn

Corporation: Ethicon Inc.

Mentor: Ashley DeAnglis

Project Title: N/A

General Objective of the Project: To characterize the extent of a crosslinking reaction.

Student’s Contribution to the Project: Developed the methods and measured the number of crosslinks per molecule.

Techniques Learned: Gel Electrophoresis, Biochemical Techniques

Student Comments on the Company and Mentor: Ethicon Biosurgery was an excellent place for a graduate student. The front end research that goes on in the department is very similar work as in graduate school. Ashley DeAnglis is an excellent scientist who thoroughly knows both the theory and technical details of many techniques. I learned something new from him everyday and learned a great deal about experimental design and analysis.
STUDENT INTERNSHIP REPORT SUMMER 2016

Student Name: Ilija Melentijevic

Department and Advisor: Microbiology and Molecular Genetics, Dr. Monica Driscoll

Corporation: Silagene

Mentor: Dr. Sam Gunderson

Project Title: Identifying therapeutic targets for the U1 adapter gene silencing platform and developing a business and marketing plan for Silagene.

General Objective of the Project: Identify orphan diseases where the Silagene's technology could be applied. Rebrand the company so that it would look more attractive for investors and donors, then develop and launch a marketing plan to raise awareness of the company's core technology and generate investor interest.

Student’s Contribution to the Project: I identified several diseases that the U1 adapter technology could be applied to, including a subset of Cystic Fibrosis cases. I also spent some time shadowing the founder and learning about the struggles and advantages of running a startup biotech company. As a background, Silagene has developed a potentially highly impactful therapeutic platform that has the potential to be applied rapidly to a large number of diseases. Developed in human cells and displaying high bio-availability and non-existent immune reaction, this platform appears to have a better chance of being translational to a human patient than other gene expression therapeutics. Despite being around for over 5 years, Silagene has had trouble establishing large pharmaceutical partnerships and finding enough funding for a clinical trial, in part due to being over-shadowed by RNAi and now CRISPR in the media. I saw an opportunity for Silagene to rebrand and leverage the gene editing hype right now to get some name recognition for its product. I reviewed the intellectual property of Silagene, which has a unique combination of primary platform patent protection, trade secrets, and the potential for independent patents on subsequent individual products made through the platform. I found a hole in their intellectual property in the form of their technology name not having a trademark. I developed the name TAILR to be used in place of the U1-adapter for its availability with the USPTO, its relative uniqueness on Google searches, and its resemblance to CRISPR. I designed a new website for the company to increase its web authority and resemble a more mature startup. I wrote out a clear marketing plan for how to utilize press releases, targeted reporter outreach, and viral distribution. I also developed an alternative business plan for the company in the case that a pharmaceutical partnership did not materialize. The plan involved the company making its product available for research applications, building awareness of the technology in the community, and bringing in funds to cover the development and validation of new products.

Student Comments on the Company and Mentor: Silagene is at an interesting stage. On one hand, the founder has managed to secure enough funding through grants to remain operational for years to come, while on the other hand Silagene has failed to secure a venture capital investment or a large pharmaceutical partnership. Dr. Gunderson was very welcoming to all of my questions, and provided me with a unique insight into the struggles with getting a startup to the next level. I think the technology Dr. Gunderson developed is incredibly powerful and translational, which makes its lack of success in commercialization heartbreaking, especially when paralleled by the rise of obvious over-hyped technology. I plan to stay involved with the company and see if I can help their technology break out.
### Fall 2016
16:125:603 Topics in Advanced Biotechnology I
Fridays, 9:30-11:30, BME Room 122

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<td>Paulina Krzyczysk</td>
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<tr>
<td>October 28</td>
<td>Industry Perspective</td>
<td>Phil Tedeschi</td>
<td>Mollie Davis</td>
<td>Jenna Newman</td>
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<td>November 11</td>
<td>Industry Prospective</td>
<td>Mehdi Ghodbane</td>
<td>Kirsten Svane</td>
<td>Eve Reilly</td>
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<tr>
<td>December 9</td>
<td>Ethics - Bioweapons (Lethal and Non-Lethal)</td>
<td>Nancy Connell</td>
<td>Jeremy Anderson</td>
<td>Christopher Rathnam</td>
</tr>
</tbody>
</table>

* Faculty presentations are limited to 30 minutes

**Trainee Presentations are limited to 10 slides and no more than 15 minutes to allow for discussion
Biotechnology Training Program: Introduction and Expectations

Maish Yarmush
16:125:603: Topics in Advanced Biotechnology I
September 16, 2016

Course Expectations
• Class is Friday from 9-11am; please be on time! (In this case please indicates a courteous demand and not a request)
• Breakfast at 8:30am
• Attend all sessions; 6 this semester
• Email Mary Ellen if conflict arises
• Deliverables: Knowledge, presentation skills, professional maturation, program-related announcements and information
• Student presentations: 10 slides + title & acknowledgements slides (no more than 15 minutes)
• To succeed you must pay attention to detail, focus on teaching, and practice, practice, practice (see Ann’s presentation on sakai from Fall 2015 for tips)

Welcome New Biotech Fellows

Alaina Howe  Sonia Yevick  Zachary Fritz
Anton Omelchenko  Larry Cheng  Christopher Rathnam

2016-17 Rutgers Biotech Fellows

<table>
<thead>
<tr>
<th>Name</th>
<th>Advisor</th>
<th>Name</th>
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</thead>
<tbody>
<tr>
<td>Alaina Howe</td>
<td>Lee</td>
<td>Christopher Rathnam</td>
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<td>Alina Howe</td>
<td>Sofou</td>
<td>Thomas Linz</td>
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<td>Anton Omelchenko</td>
<td>Firestein</td>
<td>Sunny Liew</td>
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<td>Larry Cheng</td>
<td>Drake</td>
<td>Iija Mattioli</td>
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<td>Androulaki</td>
<td>Sarah Misko</td>
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<td>Jeremy Anderson</td>
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<td>Seoul-A Bae</td>
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<td>Dan Meyers</td>
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<td>Daniel Browe</td>
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<td>Mollie Davis</td>
<td>Yarmush</td>
<td>Eann Newman</td>
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<td>Sal Ghobanne</td>
<td>Dunn</td>
<td>Evelyn Oleae</td>
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<td>Broker</td>
<td>William Pfaff</td>
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<td>Peter Scump</td>
<td>Driscoll</td>
<td>Eve Riley</td>
<td>Garcia</td>
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<td>Kate Fitzgerald</td>
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<td>Ana Rodriguez</td>
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<td>Williams</td>
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Course Requirements

<table>
<thead>
<tr>
<th>Subject</th>
<th>Credit Hours</th>
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</thead>
<tbody>
<tr>
<td>Molecular and Cellular Biology</td>
<td>3</td>
</tr>
<tr>
<td>Biophysical or Biointerfacial Chemistry</td>
<td>3</td>
</tr>
<tr>
<td>Biotechnology or Quantitative Science</td>
<td>3</td>
</tr>
<tr>
<td>Ethical Scientific Conduct</td>
<td>1</td>
</tr>
<tr>
<td>Required Courses in Graduate Discipline</td>
<td>0-12</td>
</tr>
</tbody>
</table>

Total Credit Hours (Minimum) 72

All papers are required to have PMCID #s

- PMCID #s on papers are required to track grant outcomes (http://www.ncbi.nlm.nih.gov/pmc/)
- All papers are required to have PMCID #s
- Collaborate with your colleagues to associate publications with NIH awards, and track public access compliance or declare the paper is not applicable. A video overview is available.

Recent News

June 15, 2016
Yarmush Receives NIH Grant to Develop New Interdisciplinary Statistics Course

Professors Martin Yarmush and Ramsey Foty have received a 5-year $85,910 grant from the NIH to develop a new course entitled, "Interdisciplinary Biostatistics Research Training in Molecular & Cellular Sciences: Emphasis on Rigor and Reproducibility." The course will: 1) use enquiry-based learning to strengthen understanding of statistical analysis; 2) emphasize practical rather than theoretical aspects of statistical analysis methods commonly used in the molecular and cellular sciences; 3) analyze real data generated by the student pool; 4) stress the importance of considering data analysis as a pre-requisite to experimental design; 5) teach students how to use open-source, easy-to-use statistical analysis tools.

August 20, 2016
Yarmush Receives NIH Grant to Develop New Professional Preparedness Course

Professors Martin Yarmush and Susan Engelhard have received a 1-year $84,310 grant from the NIH to develop a new course entitled, "Professional Preparedness in Biotechnology." The course will cover a myriad of topics including: the NIH and biomedical sciences ecosystem; managing communications; organizations and partnerships; project planning and management; customer focus; financial management; operations management; risk management, quality and safety; and regulatory processes.

Progress Report Schedule

- October 3: submit progress report research paragraph to advisor and lab members for editing and comments
- October 10: submit edited research paragraph along with the annual progress report form to Mary Ellen for review by Maish and Ann
- October 17: Edited progress reports returned to students
- October 26: Final progress reports due to Mary Ellen
- November 15: NIH progress report due
- December 1: IDP due to Mary Ellen
- January, February: Annual review meetings with Maish and Ann

Summer Industrial Internship

- All fellows must complete a 8-10 week summer internship during the first summer semester that they are in the program.
- Exemptions provided to those students who have previously worked for 1 year in the Biotech industry upon submission of documentation.
- All first year students must submit an application and CV to the Biotech program office by November 15 of their first year.
- An organizational meeting will be held in December to discuss student interests and potential slots in industry.
- During the spring semester the Biotech program office works together with each student to identify and match students with potential industrial mentors and sites. Final matches are made with an industrial partner based upon a number of criteria including previous research experience, specific technical skills, courses completed, and success of an on-site interview with the company.
- Fellows are required to keep the Biotech office informed of all activities related to securing an internship and participating in the internship.
### Course Requirements

<table>
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<tr>
<th>Subject</th>
<th>Credit Hours</th>
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<tr>
<td>Bioengineering/Quant Science (Statistics for Molecular &amp; Cellular Sciences)</td>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>Required Courses in Graduate Discipline</td>
<td>0-12</td>
</tr>
<tr>
<td>Bioengineering in the Biotech &amp; Pharmaceutical Industries</td>
<td>3</td>
</tr>
<tr>
<td>Innovation and Entrepreneurship for Science &amp; Technology</td>
<td>1</td>
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<tr>
<td>Topics in Advanced Biotechnology I and II</td>
<td>10</td>
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<tr>
<td>Laboratory Rotations</td>
<td>0-3</td>
</tr>
<tr>
<td>Summer Industrial Rotation</td>
<td></td>
</tr>
<tr>
<td>Graduate Research</td>
<td>39-48</td>
</tr>
<tr>
<td>Total Credit Hours (Minimum)</td>
<td>72</td>
</tr>
</tbody>
</table>

### Expectations

- Do well in 1st year courses
- Find a good thesis project
- Apply for fellowships
- Defend thesis proposal by end of year 2/beginning of year 3
- Finish in 4-6 years
- Publish (at least 2 first author papers)
- Read the literature; attend seminars
- Attend meetings/join scientific societies
  - Present your work (poster/oral presentation)
  - Network

### Acknowledgments

- Acknowledge NIH/NIGMS' full or partial support of your research in journal articles, oral or poster presentations, news releases, interviews with reporters and other communications.

> "Research reported in this ........ was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number T32 GM008339"

### Guidances

- **Approach:** Sharing versus receiving/altruism versus egoism
- These two voices are always competing
- Obstacles are growth inducers and should be met with energy and enthusiasm (butterfly example)
- Kipling: "If you can meet with triumph and disaster and treat those two impostors just the same........Yours is the Earth and everything that's in it, And—which is more—you'll be a Man, my son!"
- One earns the quality of bravery after facing the battle
- The word "sacrifice" is misunderstood today
- Sacrifice instant gratification and transform this negative behavior into sharing, proactive behavior; sacrificing ego-driven behavior for an act of humility or greed for an act of generosity.

### Character Traits

- Sharing, sacrifice, generosity, tolerance, appreciation, enthusiasm, reliability, empathy, understanding, etc.

### Skills

- Logical and critical thinking, creativity, communication (clear writing and speaking), understanding the professional culture and how to interact within it, rigor, etc.

### Brief Discussion

- Please share obstacles faced and overcome
Alginate-Encapsulated Mesenchymal Stromal Cells for the Treatment of Osteoarthritis

Ileana Marrero-Berríos
Topics in Biotechnology I
September 16, 2016

Osteoarthritis (OA)

• Slow progressive degeneration of articular cartilage.
• Affects more than 12.4 million people over the age of 65 in the U.S.
• By the year 2030, an estimated 25% of the adult population in the US will be afflicted with OA resulting in some form of disability.
• Principal source of disability resulting in increased health care costs and impaired quality of life in the U.S.
  - Healthcare cost > $185.5 billion per year

Current treatments reduce symptoms initially but are not disease-modulating and disease progression continues.

Alginate encapsulation provides a controlled delivery vehicle for MSCs that can attenuate inflammation and promote tissue repair in vivo.

eMSC modulate inflammation and secrete chondrogenic factors

• eMSC anti-inflammatory function may decrease inflammation in OA joints by down-regulating pro-inflammatory factor secretion from chondrocytes and synoviocytes.

• eMSC secrete chondrogenic factors and molecules that may promote cartilage re-growth and healing in OA joints.
To extend and promote the effect of MSC in the joint, we propose to use alginate-encapsulated human MSC (ehMSC) to:

- reduce joint inflammation and destruction characterized by OA
- promote re-growth and healing

We aim to assess the therapeutic efficacy of ehMSC by:

1. Establishing an in vitro cell culture model of OA
   - Optimizing ehMSC therapy for OA

Objectives

**Results**

- eMSCs attenuate the expression of OA mediators

**Experimental Setup**

- Monolayer chondrocytes and MSC co-culture OA model
  - +/- eMSCs & +/- IL-1α (10ng/mL)

**Inflammatory Gene List**

- Identify gene expression changes in:
  - Chondrocytes stimulated with +/- IL-1α
  - Chondrocytes +/- IL-1α and +/- IL-1α free/encapsulated MSCs

**Changes in eMSC environment induce differential secretion**

- Collect supernatants for ELISAs
- Perform LIVE/DEAD stain

**Ongoing Studies**

- 1.7% alginate eMSC show high viability and increased TGF-β1 secretion over time.
- 1.7% eMSCs might be a better treatment option for OA.

**Future Studies**

- Analyze other metrics: proliferation, collagen II and proteoglycan deposition.
- Study cell-cell interaction in a 2D and 3D tri-culture system including macrophages, chondrocytes and MSC.

Thank you!
C. elegans is useful for studying neurodegeneration. Day 1 and Day 15 Adult images are shown.

A review of the exopher.

Timing of exopher production graph.

Material can be transferred via the filament.

The exophers contain aggregated proteins.

Exophers occur in the absence of transgenes.

Calcium can flow through the filament from the soma to the exopher.

Human Aβ-42 expressed in ASER neurons causes an increase in exophers.

Heat shock mutants display elevated exopher levels.

Amphid exophers peak on day 3.
The exophers contain damaged mitochondria

- Exophers can incorporate mitochondria
- Juglone, a mitochondria-inhibiting drug, increases the number of exophers containing mitochondria
- Mitochondria in exophers are more likely to be oxidized

Mutant pdr-1 increases the number of exophers

Exophers contain lysosomes

- Neuron soma with 2 perinuclear lysosomes
- Exopher containing a lysosome
- Exophers can contain both mCherry and lysosomes

Where do exophers (and their contents) go?

- Coelomocytes can concentrate neuronal fluorescent proteins
- mCherry co-localizes to cup-4 labelled coelomocytes
- When cup-4 is knocked down, mCherry accumulates in the body cavity

What is the mechanism?

- Network of exopher hits and associated proteins
- Pared network
- High throughput genetic screen

Exopher Summary

- The exopher is a newly discovered vesicle
  - Significantly larger than other well-characterized vesicles
  - Can remain connected and can transfer material via filament
  - Can occur in physiologically relevant contexts
  - Can eject protein aggregates, mitochondria, and lysosomes
  - Contents get ingested by scavenger cells in the body cavity

Acknowledgments

Research reported in this presentation was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number T32 GM008339

Questions?
Why Are We Discussing Rigor and Reproducibility?

- NIH began focusing on rigor and reproducibility in 2014.
- New requirements have been introduced since then.
- Additional requirements will continue to be rolled out, including requirements for trainees, anticipated in 2017.

Scientists at Bayer Report Inconsistencies between Published Data and In-House Data (2011)

Believe it or not: how much can we rely on published data on potential drug targets?

Phirian Prinz, Thomas Schlange and Khursu Asadullah

Scientists at Bayer Report Inconsistencies between Published Data and In-House Data (2011)

Methods:
- Analysis of early in-house projects by questionnaire
- Comparison of in-house to published data and project outcome
- Oncology, women’s health, cardiovascular disease in recent 4 years
- Responses from 23 scientists, representing 67 projects

Results:
- 20-25% of projects had published data in agreement with in-house findings
- ~23% had inconsistencies that prolonged target validation or resulted in project termination for lack of sufficient evidence to validate the therapeutic hypothesis
Scientists at Amgen Report Irreproducibility of Preclinical Research Findings (2012)

Methods:
- Before pursuing a line of research, scientists tried to confirm published findings
- Haematology and oncology department
- 53 papers were deemed landmark studies
- Acknowledgment that papers were selected for describing something completely new

Results:
- Scientific findings were confirmed in only 6 of 53 cases (11%)
NIH Leadership Responds (2014)

- Acknowledged problem
  - The issue is greater for preclinical than clinical research (highly regulated)

- Proposed steps that NIH will take immediately
  - Develop required training module incorporated into ethical conduct (intramural)
  - Pilot checklist for systematic evaluation of grant proposals
  - Develop Data Discovery Index to access unpublished primary data

- Emphasized need for community engagement
  - Scientific publishers (methods, primary data, statistical analyses during review)
  - University tenure and promotion committees should emphasize quality over quantity
  - NIH Biosketch reformatted to emphasize scientific contributions, not # of papers

NIH Website: Resources for Rigor and Reproducibility

NIH Introduces New Requirements for Grant Applications (2016)

“NIH’s Rigor and Transparency efforts are intended to clarify expectations and highlight attention to four areas that may need more explicit attention by applicants and reviewers:”

- Scientific premise
- Scientific rigor
- Consideration of relevant biological variables, such as sex
- Authentication of key biological and/or chemical resources

Review Criteria for Rigor and Transparency of Research (R01 Grants)

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Applies to which applications?</th>
<th>Where is it included in the application?</th>
<th>Addition to review criteria</th>
<th>Affect overall impact score?</th>
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</thead>
<tbody>
<tr>
<td>Scientific Premise</td>
<td>All</td>
<td>Research Strategy (Significance)</td>
<td>Is there a strong scientific premise for the project?</td>
<td>Yes (Significance)</td>
</tr>
<tr>
<td>Scientific Rigor</td>
<td>All</td>
<td>Research Strategy (Approach)</td>
<td>Are there strategies to ensure a robust and unbiased approach?</td>
<td>Yes (Approach)</td>
</tr>
<tr>
<td>Consideration of Relevant Biological Variables, Such as Sex</td>
<td>Projects with vertebrate animals and/or human subjects</td>
<td>Research Strategy (Approach)</td>
<td>Are adequate plans to address relevant biological variables, such as sex, included for studies in vertebrate animals or human subjects?</td>
<td>Yes (Approach)</td>
</tr>
<tr>
<td>Authentication of Key Biological and/or Chemical Resources</td>
<td>Project involving key biological and/or chemical resources</td>
<td>New Attachment</td>
<td>Comment on plans for identifying and ensuring validity of resources</td>
<td>No</td>
</tr>
</tbody>
</table>
Scientific Premise

**GOAL:** Ensure that the underlying scientific foundation of the project (concepts, previous work, and data, when relevant) is sound.

- Pertains to the underlying evidence/data for the project
- Address under Significance (R applications)
- Addition to the review criteria: "Is there a strong scientific premise?"
- Specifically, has the applicant:
  - Provided sufficient justification for the proposed work?
  - Cited appropriate work and/or preliminary data?
  - Appropriately identified strengths and weaknesses in prior work in the field?
  - Proposed to fill a significant gap in the field?
  - OR has the applicant explained why this is not possible?

Relevant Biological Variables

**GOAL:** Ensure that the research accounts for sex and other relevant biological variables in developing research questions and study designs. The ways in which sex and other biological variables need to be accounted for will differ across research questions and fields of study.

- Pertains to the proposed research (vertebrate animals, human subjects)
- Address in Approach (R applications)
- Addition to review criteria: Are there "adequate plans to address relevant biological variables for studies in vertebrate animals or human subjects?"
- Consideration of sex is required in all studies involving human subjects or vertebrate animals.
- Specific considerations to assess include:
  - Applies broadly to all biological variables relevant to the research such as sex, age, source, weight, or genetic strain.
  - Has the applicant considered biological variables, such as sex, that are relevant to the experimental design?
  - Will relevant biological variables be controlled or factored into the study design appropriately?

Plan for Resource Authentication

**GOAL:** Ensure processes are in place to identify and regularly validate key resources used in their research and avoid unreliable research as a result of misidentified or contaminated resources.

- Researchers are expected to authenticate key biological and/or chemical resources used in their research, to ensure that the resources are genuine.
- New additional review consideration: "Authentication of Key Biological and/or Chemical Resources. For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources."
- Does not affect criterion scores or overall impact score (rated as acceptable or unacceptable)

Will these Steps Enhance Reproducibility?

Awareness of the problem is an important first step.

- Reproducibility is being studied, and research published
- New initiatives raise awareness
  - Training
  - Journal standards
  - NIH grant application criteria
- However, a major cultural change may be required for sustained impact.
Pressure to Publish Selects for Irreproducibility

The natural selection of bad science

Paul E. Smaldino1 and Richard McElreath2
1Department of Biology, University of California, Irvine, CA 92697, USA
2Department of Computer Science, University of California, Irvine, CA 92697, USA

"Poor research design and data analysis encourage false-positive findings. Such poor methods persist despite perennial calls for improvement, suggesting that they result from something more than just misunderstanding. The persistence of poor methods results partly from incentives that favour them, leading to the natural selection of bad science."

Published 21 September 2016

Institutional Incentives for Scientific Researchers

- Increases in publication rate
  - average puts of newly hired biologist: 22 in 2013, 12.5 in 2005
- Pressure to portray work as groundbreaking
  - 25-fold increase in "innovative", "groundbreaking" and "novel" in PubMed abstracts
- Overuse of h-index
  - Researchers are rewarded for publications
  - Positive results are easier to publish and more prestigious than negative results

Researchers who can obtain more positive results (whatever their truth) will have an advantage

Published 21 September 2016

An Evolutionary Model of Science

- Each lab has a characteristic power, the ability to positively identify a true association.
- Increasing power also increases the rate of false positives, unless effort is exerted.
- Increasing effort decreases the productivity of a lab, because it takes longer to perform rigorous research.
- Labs receive "pay-offs" for publishing their research (prestige, funding, etc.; can be positive or negative)
- Labs die randomly and those with higher pay-offs reproduce. Labs inherit attributes of their parent lab (but with mutation probabilities).

Published 21 September 2016
An Evolutionary Model of Science

Simulation with Constant Effort
As power increases, the rate of false positives increases.

Effort Evolves
As effort decreases, the rate of false positives increases.

A Cultural Change is Required to Promote Reproducibility

Science is a cultural activity and can change through evolutionary processes. Incentives drive cultural evolution.

"Some of the most powerful incentives in contemporary science actively encourage, reward and propagate poor research methods and abuse of statistical procedures."
Integration of genomic, transcriptomic, and proteomic information into PK/PD models: A case study in methylprednisolone

Alison Acevedo
Biomedical Engineering Department, Rutgers University
Advisor: Dr. Ioannis Androulakis

Methylprednisolone: anti-inflammatory and immunosuppressive therapy
• MPL: synthetic glucocorticoid (corticosteroid) similar in structure to cortisol
• Anti-inflammatory agent and immune suppressant
• CS action in the liver – liver is responsible for glucose storage, gluconeogenesis, lipid storage, etc.
• Chronic use yields adverse metabolic effects: hyperglycemia, dyslipidemia, arteriosclerosis, muscle wasting, etc.
• Seek to understand how these adverse effects manifest.
• Investigation is generalizable

PK/PD investigation of MPL dynamics
Mechanisms of action of corticosteroids
Pharmacokinetic/Pharmacodynamic (PK/PD) analysis describes how a drug is affected after administration and the time course of the effects that the drug has on the subject.
Adverse effects tend to manifest with chronic CS application.
Adverse effects manifest via metabolic pathways, therapeutic effects via immune pathways – both mediated by glucocorticoid receptor (GR).
Chronic use of CS yields sustained downregulation of GR, however drug effects remain potent – indicative of alternative mechanisms of CS action than simple GRE binding. (Ngoenh, BMJ 2010).

Temporal effects reveals expression of immune and metabolically relevant mRNA and protein.
• Rat MPL dosing studies for proteomic and transcriptomic data
  – 60 ADX male Wistar rats, single MPL bolus, sacrificed at 12 time points between 30 min and 66 hr – MS/LC for protein quantification (Nouri-Nigjeh, Anal Chem. 2014)
  – 43 ADX male Wistar rats, single MPL bolus, sacrificed at 16 time points between 30 min and 72 hr – mRNA expression quantified via microarray (Jin, J Pharm Exp Therapeut. 2003)
• Cluster and functional analysis of concatenated temporal transcriptomic and proteomic data reveal immune and metabolically relevant dominant genes
  – Parallel and anti-parallel correlation reveals more complex dynamics than simple correlation of transcription with translation

Preliminary model developed to describe liver response to MPL
• Proof-of-concept model developed using six genes functionally related to representative pathways
• Dynamics initiated by GR in the nucleus (DRN) affecting all transcription targets
• Transcriptional events further modulated by protein interaction with transcription factors

Expand PK/PD to Reflect System Complexity
Combination of established MPL PK/PD with an expanded understanding of transcriptomic and proteomic events is possible via omics analysis for capturing temporal changes in mRNA and protein in response to MPL dose (Kamisoglu, OMICS. 2015).
Preliminary results partially capture protein dynamics.

Initial assumption: transcription rate is controlled by the activity of regulatory proteins and translation is proportional to mRNA.

Data trends challenge this assumption.

Model construction guided by search for means of generating these protein dynamics.

Summary

- Seek to understand the therapeutic and adverse effects of synthetic corticosteroids.
- Ultimately seek to develop model generalizable to other drugs to further understand and predict system response.
- Currently developing a system which reflects molecular dynamics captured through omics analysis specific to MPL in the liver.
- Network construction informed by collected genomic, transcriptomic and proteomic data.
- Current model effective in capturing some system dynamics and requires further analysis and expansion to reflect complexity of actual MPL response.

Dynamics can be further captured with...

Non-linear effects remain to be captured: feedback, saturation effects, protein-protein and protein-transcript interactions, alternate transcription factor dynamics.

Integration of precursor dependent model (Jusko 2013) in grmt1/GSTM1 system.

Network construction accuracy improved with integration of genomic, transcriptomic and proteomic data.

Acknowledgments

BME Rutgers University
Seul-A Bae, Rohit Rao, Kamau Pierre, Clara Hartmenschen, Meg Escotet, Jonathan Pai (graduated)

Pharmaceutical Sciences,
SUNY Buffalo
Kubra Kamisoglu
Richard Almon
William Jasuko
Debra Dubois

Biotech Training Program
This work was supported in part by both the NIH Biotechnology Training Program (Award T32 GM008339) and the NIH GM024211.


Removal of Slides

Mathematical notation used to represent the product of terms

Reminder of Pi Notation

Relevant for the construction of models in Meister and Bintu
A 3D PRINTED POLYMER-REINFORCED COLLAGENOUS SCAFFOLD FOR PARTIAL MENISCUS REGENERATION
Sal Ghodbane

Advisors: Michael Dunn, Ph.D. Charles Gatt, M.D.

Knee Menisci

- Functions
  - Joint stability
  - Shock absorption
  - Lubrication
  - Load Transmission

- Semi-lunar fibrocartilage structures with a concave proximal surface and flat distal surface
- Attachments
- Meniscal injuries are one of the most common orthopaedic injuries
- 700,000 partial meniscectomies performed in US per annum ($4 billion market)
- Partial meniscectomies have excellent clinical outcomes in the short term up to 4 years
  - Jaureguito et al. found that at 8 year follow-up excellent or good outcomes dropped from 92% to only 68%
- Long term deterioration
  - Direct correlation between extent of meniscectomy and onset of osteoarthritis

Fibrocartilage

Ligament  Cartilage  Ligament Fibrocartilage Cartilage

Tension  Compression and Shear  Compression and Shear

Ideal Partial Meniscus Scaffold

- Anatomical Requirement
- Biological Requirement
  - Cell attachment, infiltration, and distribution
  - Transport of nutrients and signals
- Mechanical Requirement
  - Possess appropriate mechanical properties to provide the correct mechanical loading environment for infiltrating cells
  - Protect the underlying articular surfaces
- Clinical Requirement
  - Can be cut to unique defect geometry
- Can not pre-determine exact defect geometry using imaging
- Fixation that allows for mechanical function and tissue integration

Partial Meniscal Implants

- No FDA approved synthetic meniscus replacement device

<table>
<thead>
<tr>
<th>Material</th>
<th>Matrix Orientation</th>
<th>Total/Partial Meniscus</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actifi</td>
<td>Porous PCL, isotropic</td>
<td>Partial</td>
<td>Inner rim is worn away before infiltration (correct load bearing tissue)</td>
</tr>
<tr>
<td>Menaflex</td>
<td>Porous Collagen (glutaraldehyde)</td>
<td>Isotropic</td>
<td>Partial</td>
</tr>
<tr>
<td>Abilgene</td>
<td>Decellularised ECM</td>
<td>Anisotropic</td>
<td>Total</td>
</tr>
</tbody>
</table>

Results
**Project Goal**

- Total Meniscus Scaffold
  - Composed of 100 µm single woven polymeric fiber
  - Collagen Sponge
  - Tissue inductive and conductive
  - Chondroprotective up to 52 weeks
- Poly(DTDDD) reinforcement
  - Tyrosine derived polyarylate
  - Does not produce acidic degradation products

The goal of this project is to adapt this technology to partial meniscus defects. Therefore, there is a need for a manufacturing method that can produce a geometrically complex and interconnected structural reinforcement.

We hypothesize that a 3D printed polymer reinforced collagenous scaffold can be fabricated and implanted to:
1. Mimic the mechanical anisotropy of the native meniscus
2. Be successfully fixed to the remaining native meniscal rim
3. Increase the contact area and reduce peak stresses relative to partial meniscectomy
4. Encourage cell infiltration, extracellular matrix production, and organized tissue deposition
5. Integrate robustly to the surrounding native meniscal tissue, and
6. Protect the articular surfaces to prevent or delay degenerative changes in the underlying cartilage.

**Proof of Concept Studies**

- Radial Layers
- Circumferential Layers

**Mechanical Optimization**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micromechanical size</td>
<td>150-600 µm</td>
<td>Promotes fibrocartilage ingrowth1</td>
</tr>
<tr>
<td>Circumferential Tensile Modulus</td>
<td>40.75-57.4 MPa</td>
<td>Avoid catastrophic tensile failure2</td>
</tr>
<tr>
<td>Circumferential : radial tensile stiffness (anisotropy)</td>
<td>6.4-13.8</td>
<td>Promotes fibrochondrocytic phenotype3</td>
</tr>
<tr>
<td>Aggregate Compressive Modulus</td>
<td>&gt;150 kPa</td>
<td>Distribute peak contact stresses4</td>
</tr>
</tbody>
</table>

**Specific Aim: Mechanical Optimization of 3D Printed polymer architecture to approach the anisotropic tensile mechanics of the native meniscus.**

- Circumferential Tensile Evaluation
  - Scaffold tensile properties compared to 80% inner margin
- Radial Tensile Evaluation
- Regional Evaluation of Confined Compression Creep
  - Mow’s biphasic theory
- Poly(DTDDD) Degradation Assay

**Specific Aim: Develop a surgical procedure that will produce suitable fixation that allows for tissue ingrowth and integration and improved functional biomechanics than simple suture techniques.**

- Scaffold Body Single Suture Pull-out Study (Suturability)
- Scaffold-Native Meniscus Construct Pull-out Study
- Scaffold Contact Stress Study

2. Verdonk et al. 2011
3. Tissakht et al. 1995
In Vitro Contact Stress Study
- Simulate the contact stresses imposed by the ovine femoral condyle onto the tibial plateau
  - Intact meniscus
  - Partial meniscectomy
  - Implanted partial meniscus scaffold
  - Total meniscectomy
- Each condition is tested in 6 ovine knee joints
- High peak contact stresses and reduced contact areas have been shown to cause the onset of osteoarthritis

In Vivo Evaluation
- Determine the extent that the partial meniscus scaffold can
  - promote neo-fibrocartilaginous tissue ingrowth
  - preserve the underlying articular surfaces of the knee joint
  - integrate with the remaining native meniscal tissue
- Posterior partial meniscectomies
  - 8, 16, and 32 weeks (n=3/time point)

1. Confined Compression Creep
2. Tensile Integration Strength Testing
3. Histology of the Explant and Cartilage
4. Immunohistochemistry of the explant
5. Biochemical Analysis of the explant
6. Synovial Fluid ELISA

Acknowledgements
- Biotechnology Training Program Fellowship
- Orthopaedic Research Laboratory
  - Jay Patel
  - William Pfaff
- Center of New Jersey Biomaterials
  - Joe Modic
- Michael Durin, Ph.D.
- Charles Gatt, M.D.
- The Armed Forces Institute of Regenerative Medicine (AFIRM II)

Questions

References
- DeHaven, K., et al. Development of a meniscal meniscal meniscal meniscus meniscal meniscus meniscus meniscal meniscal meniscal meniscus meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal meniscal 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- pull-out and shear failure strengths of arthroscopic meniscal repair systems
- 2. Finite element analysis of the meniscus: the influence of geometry and material properties on its behaviour.
- 5. Interdependent load-bearing properties of meniscus and articular cartilage: implications for meniscal repair.
- 6. Synovial Fluid ELISA
- 7. Immunohistochemistry of the explant
- 8. Histology of the Explant and Cartilage
- 9. Confined Compression Creep
- 10. Tensile Integration Strength Testing
Aim 1: Collagen Sourcing Issues

- Collagen sponges provide a structural template for cells to infiltrate, adhere, and replace with neo-meniscal tissue.
- Bovine collagen is a primary source for medical devices.
- Bovine derived collagen carries the risk of transmitting bovine spongiform encephalopathy.
- We hypothesize that collagenous sponge-like scaffolds isolated from porcine and ovine tendons possess comparable mechanical and enzymatic properties as those derived from bovine tendons.

In comparison to bovine scaffolds, ovine scaffolds performed equivalently or superiorly in all evaluations and porcine scaffolds were equivalent in all properties except enzymatic stability.

References:
- Patel et al. 2016 Journal of Tissue Engineering
- Patel et al. 2016 American Journal of Sports Medicine
- Lee et al. 2014 Science Translational Medicine
- Ghodbane et al. 2015 Journal of Biomedical Materials Research
Fellowship Application
**SURVIVAL TIPS**

To Make the Process Less Painful
Than it Has To Be

Paulina Krzyszczyk & Alvin Chen

---

**It may seem overwhelming, especially with classes and experiments, so your first question may be...**

1. Fellowship stipends typically range from $25,000- $35,000/yr
2. Some up to 5 years!
3. They can include conference travel funds as well!

---

**WHY BOTHER?**

- They also don’t look too shabby as a line on your CV
- It’s a relief to secure long-term funding

---

“Grant-writing is a form of thinking”

Dr. Martin Yarmush

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77
**Fringe Benefits**

- Flesh out your project/proposal!
- Practice writing!
- Gain a deeper appreciation for what professors are actually doing when they say they are “grant-writing”
- Learn how to “brag about yourself”, which **you will need** in your future career search!

**When should I start the application process?**

**NOW!**

I’m talking to YOU, Procrastinators!

**How do I start the application process?**

1) Find a fellowship that you are eligible for

- **MAKE SURE YOU ARE ELIGIBLE!**
  - Year in grad school (Early Career/All But Dissertation)
  - Diversity Fellowships
  - Topic Specific
- Read the application package and understand the submission process!

2) Break out your calendar!

- Mark the deadline
- Have your final draft ready several days before the final deadline

**Deadlines**

- **DOD SMART**—Dec 1, 2016
- **NSF GRFP**—Oct 24-28 (depends on research field)
- **Ford Fellowship**—Nov 17 (Pre-doctoral)
- **Paul & Daisy Soros Fellowship for New Americans**—Nov 1
- **NIH F31**—April 8, August 8, December 8
- **Gilliam Fellowships for Advanced Study**—February 2, 2017
- **Ford Fellowship**—November 10 (dissertation)
- **PEO Scholarship**—November 20
- **Bevier Scholarship (Rutgers)**—February
- **NJ Commission on Cancer Research**—October 21, 2016
- **NJ Commission on Brain Injury Research**—October 5, 2016
- **American Foundation for Pharmaceutical Education**—December
- **Pharmaceutical Research and Manufacturers of America (PhRMA) Fellowship**—September 1 or February 1 (depending on subfield)
Typical Application Components

• Personal Statement
  – Why do you love science?
  – Past research experiences
• Research Plan
  – Overall goals and hypothesis
  – Details of the proposed research
  – Preliminary Results
• Reference Letters
• Supplementary Documents

Defining Your Research Plan

• Problem and Significance (Why do I care?)
  • What is the scientific or health problem?
  • What is the gap in current knowledge/methods/tools?
  • Why is this a critical gap to fill?
  • What is the rationale behind your approach?

• Central Hypothesis
  • Specific question you propose to ask
    (not a general examination of a topic)
• Key Research Objectives (2-3)
  • Each objective should test the central hypothesis
  • Each objective should result in a measurable outcome

Create a one-paged outline (called your Specific Aims)

Three C’s

• Completeness
  • No missing sections!
  • Pay attention to details!
• Clarity
  • Audience: smart scientists, but may not be in your sub-field
  • Minimize jargon, use active voice, format appropriately
  • Make use of figures and graphics
  • Write many drafts, and use outside readers
• Coherence
  • Tie your entire application around some central points
  • Hammer in your main strength/passion via repetition
  • Application should be a portrait of YOU, not the project

Reference: https://www.pgbovine.net/fellowship-tips.htm

Applying is a Team Effort!

• Set up meetings and request items from others early!
  o With your advisor/committee members
    ➢ Develop an application strategy
    ➢ Past, related applications
    ➢ Reference letters
    • Don’t forget your past connections!
  o Grants specialists
    ➢ Department specific
    ➢ https://orsp.rutgers.edu/rutgers-gsstaff-nbpisc
    ➢ Understand the application process…the F31 is extensive and confusing!
    ➢ eRA Commons account
    ➢ Internal documents
    • They ultimately submit your application
• GradFund
  • http://gradfund.rutgers.edu/
• Graduate Students
  – Especially those who have successfully applied before!

Biotech Mentors

• NIH F31—Alvin Chen, Paulina Krzyszczyk
• NSF GRFP—Seul-A Bae, Evelyn Okoke, Antoinette Nelson, Daniel Browe
• NJ Commission on Cancer Research—Sarah Misenko, Dharm Patel, Thomas Linz
• NJ Commission on Brain Injury Research—Christopher Lowe, Kate O’Neill, Peter Swiatkowski
• Ford Dissertation Fellowship—Kate O’Neill
• PEO Scholarship—Kathryn Drezewicki
• Gilliam Fellowships for Advanced Study—Ilesana Marrero-Barrios
• American Foundation for Pharmaceutical Education—Antoinette Nelson
• Pharmaceutical Research and Manufacturers of America (PhRMA) Fellowship—Dan Myers

NSF GRF
(National Science Foundation Graduate Research Fellowship)

www.nsfgrfp.org
**NSF GRF**

- **Mission:** Support basic and applied STEM research with strong potential to advance knowledge and benefit society.
- **Eligibility:** At time of application
  - be in 1st year of graduate school, or
  - 1st semester of second year
- **Deadline:** October 24-28 (depends on research field)
- **Award:** 3 years, tuition ($12K/yr) and stipend ($32K/yr)
- **Note:** Everything centered around the Merit Review Criteria

**Merit Review Criteria**

1. **Intellectual Merit**
   - Potential of applicant to advance knowledge in the field of study
     - Show technical proficiency through past research experiences and a strong (well-defined) research plan
     - Ideally, connect your research topic to your past experiences

2. **Broader Impact**
   - Potential of applicant to benefit society, by:
     - effectively integrating research and education
     - encouraging diversity
     - enhance scientific and technical understanding
     - benefit society

**Application Sections**

1. Personal Background and Future Goals (3 pages)
2. Graduate Research Plan (2 pages)
3. Reference Letters (3 letters)
4. Electronic Transcripts
5. GRE's no longer required!

Where you've been  Where you are now  Where you want to be

**NJ Department of Health**

(Graduate Fellowships for Commissions on Spinal Cord Injury, Brain Injury, and Cancer Research)

**Mission:** Support research into causes, prevention, and treatment of specific injury/disease

**Eligibility:** Post-qualifier (dissertation research stage)

**Deadline:**
- NJ Commission on Brain Injury Research—October 5, 2016
- NJ Commission on Cancer Research—October 21, 2016

**Award:** 3 years, $22-30K/yr

**Note:** Requirements may vary between Commissions

**Application Sections**

1. Project Abstract
2. Research Plan (up to 8 pages)
3. Resources and Environment (up to 2 pages)
4. Personal Statement (1 page)
5. Reference Letters (up to 5 letters)

This is mainly a research grant
NIH F31
(National Institutes of Health Individual Predoctoral Fellowship)

https://researchtraining.nih.gov/programs/fellowships/F31

Mission:
- Enable predoctoral students to obtain individualized, mentored research training from outstanding faculty sponsors
- Enhance the recipient's potential to develop into an independent research scientist

Eligibility: Post-qualifier (dissertation research stage)

Deadlines: April 8, August 8, December 8 (5:00 PM EST)

Award: 2-4 years, full tuition and stipend (~$23K)

Note: 33% individual fellowship
33% training grant
33% research grant

Application Sections
1. Applicant’s Biosketch (Personal Statement, CV, and Transcript)
2. Doctoral Dissertation and Other Research Experience (2 pages)
3. Goals for Fellowship Training and Career (1 page)
4. Reference Letters (up to 5 letters, not including Sponsor)
5. Selection of Sponsor and Institution (1 page)
6. Respective Contributions (1 page)
7. Training Activities Planned Under this Award (1 page)
8. Environment, Facilities, and Equipment
9. Sponsor Information, including Reference Letter (6 pages)
10. Project Abstract
11. Specific Aims (1 page)
12. Research Plan (6 pages)
   a. significance and impact
   b. preliminary studies
   c. research design and methods
   d. human subjects and vertebrate animals
   e. literature cited
13. Appendix (can include “unofficial” letters of endorsement here)

Review Criteria

<table>
<thead>
<tr>
<th>Score</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 9</td>
<td>Fellowship Applicant</td>
</tr>
<tr>
<td>1 – 9</td>
<td>Sponsors and Collaborators</td>
</tr>
<tr>
<td>1 – 9</td>
<td>Research Training Plan</td>
</tr>
<tr>
<td>1 – 9</td>
<td>Training Potential</td>
</tr>
<tr>
<td>1 – 9</td>
<td>Institutional Environment</td>
</tr>
<tr>
<td></td>
<td>Overall Impact/Merit</td>
</tr>
</tbody>
</table>

“An fellowship application has a research project that is integrated with the training plan. The application emphasizes the applicant’s potential for an independent scientific research career, the applicant’s need for the training, and the degree to which the research project and training plan, the sponsor(s), and the environment will satisfy those needs.”

Links to Resources – General Tips

- General grant preparation
  - Preparing and submitting a competitive external graduate fellowship
  - Advice for writing Grants, fellowships, and personal statements
  - Advice for science fellowship applications (with specifics on NSF, NDSEG, Hertz)

- NDSEG
  - Notes for writing a successful NDSEG fellowship application

- NSF GRFP
  - NSF GRFP advice and examples

- NIH F31
  - Advice on writing NIH F31 applications from real NRSA reviewers
  - NIH F31 application walkthrough, writing advice, and review criteria

Links to Resources – Samples from Past Winners

- NSF GRFP
  - Link 1 (Sample research proposals and personal statements from 43 past winners)
  - Link 2 (More samples from numerous past winners, not all STEM)

- NDSEG
  - Link 1 (NDSEG winning essay, computational biology)
  - Link 2 (NDSEG winning essay, physics)
  - Link 3 (Comparison of winning and non-winning essays, computer science)

- NIH F31
  - Link 1 (4 full application samples from previous NIGMS winners)
  - Link 2 (F31 Diversity, 2 full application samples from NAAD winners)
  - Link 3 (research plan sample (12 pages), fatty acid metabolomics)
**Additional Tips As You Apply**

- Make the library your second home
- Take breaks! We are not robots...

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**When you have submitted it... make sure you celebrate!!!**

But don’t forget about post-submission materials and to check back for your result and feedback!

---

**Don’t get discouraged!**

- Most fellowships allow for resubmissions!
- The feedback that they provide helps dramatically improve your application and increase your chances for success!

---

**If you enjoyed this talk/topic/advice check out:**

*The Rutgers iJOBS Blog*

Please, *share* your advice and experiences!

*Any questions?*
Modulating cerebrospinal fluid to increase drug penetration into the brain using a refillable implanted reservoir

Sonia Yevick
Sy Lab
Topics in Advanced Biotechnology I
October 14, 2016

Glioblastoma Multiforme – poor prognosis

<table>
<thead>
<tr>
<th>Grade (WHO)</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (low grade)</td>
<td>Pilocytic Astrocytoma</td>
<td>Slow-growing, noninvasive</td>
</tr>
<tr>
<td>II (low grade)</td>
<td>Diffuse Astrocytoma</td>
<td>Slow-growing, invasive</td>
</tr>
<tr>
<td>III</td>
<td>Anaplastic Astrocytoma</td>
<td>Form projections into surrounding tissue</td>
</tr>
<tr>
<td>IV</td>
<td>Glioblastoma Multiforme</td>
<td>Most aggressive, malignant, often necrotic</td>
</tr>
</tbody>
</table>

- Incidence: about 50% of primary malignant brain tumors
- Prognosis: 2-year median survival = 27%
- Treatment:
  - Resection
  - Radiation
  - Chemotherapy (oral, IV or wafer)
    - Temozolomide, Carmustine (Gliadel)

Gliadel wafers – not so effective

- Rapid CSF turnover → rapid drug clearance
- Slow diffusion through the brain
  - Tortuosity + dead ends
  - Obstructions
  - Cell receptor binding
  - Negative charges – affecting diffusion of charged molecules

Increasing drug penetration

- Convection Enhanced Delivery (CED)
  - Positive pressure pushes drug into brain
- CSF modulation:
  - Lack of diffusion? Verapamil (CSF production)
  - CSF clearance? Acetazolamide (CSF production)

Ommaya Reservoir – a platform technology

- Advantages:
  - Small amounts of drug locally, consistently over time vs. bolus systemically
  - No need for multiple surgical injections
  - Administer different drugs/multiple doses over time
- Challenges:
  - Redesign for rats
  - Biocompatibility (micromotion, inflammation, immune encapsulation/scarring)

Brain image: https://indianmedtrip.com/treatments/glioblastoma-brain-tumors-treatment/
microOmmaya Reservoir

- Redesigning for in vivo studies:
  - Scale down
  - New materials
  - New design
  - Requires all new tests

in vitro – cytotoxicity

IC50 values

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Verapamil</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>400µM</td>
<td>150µM</td>
</tr>
<tr>
<td>RG2</td>
<td>150µM</td>
<td>7µM</td>
</tr>
</tbody>
</table>

Representative plots for C6 cells:

- CSF modulating drugs
  - Verapamil
  - Acetazolamide

- Chemotherapy drugs
  - Temozolamide
  - Doxorubicin

Future work

- Temozolomide at higher concentrations and longer time periods
- Fabricate microOmmaya device - testing
- in vivo work
  - tumor model (rat)
  - concurrent chemo + CSF modulator injection
  - implanted microOmmaya drug delivery (concurrent chemo + CSF modulator)
- Catheter biocompatibility

References

- Phuphanich S. Glioblastoma and Malignant Astrocytoma. American Brain Tumor Assoc. 2016, brochure
- https://www.flickr.com/photos/littoraria/ 782148275/in/photostream/

Acknowledgements

Thank you!

The Sy Lab:
- Jay Sy
- Gemi Dessi
- Rashmi, Venky, Sruthi and Allan

The Biotechnology Training Program:
Research reported in this presentation was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number T32 GM08339
Detecting Cancer Early With Microfluidics and Protein Interaction Modeling

Zachary Fritz
Martin Yarmush, Lawrence Williams
10/14/2016

• p53/TP53 - Tumor suppressor protein
• Oncogenic mutations can lead to overexpression and accumulation of p53
• Most common mutation (>50%) in a variety of cancers: lung, breast, colorectal, ovarian, esophageal
• Mechanism of extracellular p53 release unknown, probably from necrosis
• Pedersen et al. found elevated (above 95th percentile of the control) levels of p53 autoantibodies in 26% of colorectal cancer patients in a large (>50,000 women) study
• Autoantibodies could be detected years before formal diagnosis (median: 1.4 years)
• Custom multi-antigen microarray was more accurate than standard p53 ELISA in predicting cancer

Autoimmune Response to p53

Problem:
Pedersen study used crude, flawed methodology to select antigens
– Ignored whole-protein, contextual, and structural effects that could contribute to antigenicity
– Ignored mutational effects (stabilizing and destabilizing)

Goal:
Create a device and methodology that allows for the ultrasensitive detection of p53 autoantibodies, improves upon Pedersen’s results (>26% detection ability), and can adapted to other biomarkers

Pros:
– Uses smaller sample and reagent volumes
– Adds in convective transport of analytes
– Already designed and optimized (for cytokines)
– Able to run multiple samples
– Potential for automation

Cons:
– Long process time: ~7 hours
– Requires off-chip analysis (Bio-Plex 200)

Proposed Device Modifications

To reduce assay time:
– Use fewer microbeads/smaller sample volumes
– Combine detection Ab with fluorescent tag to eliminate a step
– Add optical analysis capability on-chip

Conclusion:
Comparable LODs. Device was unoptimized for p53 antibodies. Same antibodies and antigens were used in both assays, unless indicated.
**Rutgers Protein Energetics Model**

- Protein folding-unfolding is a phase transition
- Exhibits scale invariant behavior
- Power Law discovered by Moret and Zebende (2007)

**Our Model**

- Each AA is assigned an energy ($S_i$) value based on:
  - The AA's identity: an intrinsic scale invariant term
  - The secondary structure it's part of: alpha helix, beta sheet, turn
  - The identity of its "symmetrical" neighbors (structure determines symmetry)
- Use primary sequence and crystallographic structure data from the PDB
- Also get a term ($f$) to describe energy of whole protein
- Can compare $f$ to individual $S_i$ values to assess hydrophobicity of a residue

---

**Rutgers Research Nexus**

**Our Model**

- Antibodies to an isolated peptide do not recapitulate whole protein effects
- A segment that may be highly antigenic within the whole protein could be substantially less so as an isolated peptide
- Our model gives us a quantitative assessment of this: Amount of variance between protein and peptide $S_i$'s
- Flanking sequences may improve a peptide’s antigenicity
- Will use known high and modest affinity WT and mutant antigens
- Verify model’s findings with patient sera experiments

---

**Rutgers Myoglobin**

- Underlying principle: antigens should recapitulate whole protein effects
- A segment that may be highly antigenic within the whole protein could be substantially less so as an isolated peptide
- Our model gives us a quantitative assessment of this: Amount of variance between protein and peptide $S_i$’s
- Flanking sequences may improve a peptide’s antigenicity
- Will use known high and modest affinity WT and mutant antigens
- Verify model’s findings with patient sera experiments

---

**Rutgers Antigen Presentation: MAPs**

- Antigens will be immobilized onto 6 μm microbeads
- Want 10^6 - 10^7 antigens per bead
- We will synthesize branched Multi-Antigen Peptides (MAPs) via solid supported peptide synthesis
- MAPs conjugated to beads via CuII chemistry: azide + DBCO

---

**Rutgers Summary**

- We want to create a powerful diagnostic tool for detecting cancer early
- Has the benefits of a microfluidic device
- On-chip analysis
- Very sensitive: low pg/ml detection limit
- Captures autoantibodies that other assays might have missed/overlooked
- Detects p53 autoantibodies in more than 26% of current and future cancer patients
**Rutgers** Future Work

**Short Term**
- Review microfluidic flow cytometry literature
  - Write review article
- Be trained on soft lithography/replica molding
- Continue learning peptide synthesis
- Determine $k_r$ and $k_{off}$ for antibody-antigen binding using surface plasmon resonance

**Long Term**
- Select optimized antigens using model
- Design and build optically-integrated microfluidic device
- Run experiments using cancer patient sera

---

**Rutgers** Acknowledgements

- I'd like to thank:
  - Dr. Martin Yarmush
  - Dr. Lawrence Williams
  - Dr. Zoltan Szekely
  - Dr. Rene Schloss
  - Dr. And Shao
  - Yarmush and Bethiaume labs
  - The Biotechnology Training Program

---

**Rutgers**

نواعیت:
- ویژه‌سنجی
- مایع‌سنجی
- پرمایش

اینفوگرافیک:
- گرافیک مقیاس‌گذاری
- گرافیک مقیاس‌گذاری

---

**Funded by**
The National Institute of General Medical Sciences (NIGMS)
NIH T32 GM008339
Industry Perspectives: Biosimilar Development

Outline

My Background

- Worked with Joseph Bertino, MD at the Cancer Institute of New Jersey
  - Translational oncology, small molecules
- Received PhD in Molecular Pharmacology, Sept. 2011-April 2015
  - Biotechnology Training Program Fellow
  - Twelve publications
- Thesis: "NAD+ Kinase as a Therapeutic Target in Cancer"
- Previously:
  - Associate Scientist at a CRO
  - MS, The Johns Hopkins University
  - BS, The College of New Jersey

NADPH is critical in cell metabolism

- NADP+ in its reduced form (NADPH) is required for the biosynthesis of fatty acids, nucleic acids, steroids and other anabolic reactions
  - Proton acceptor in the PPP (reductive equivalents, nucleic acid and aromatic amino acids)
  - Also important for protection against oxidative stress
    - Reduces glutathione

NAD Kinase is very important

What is a Biosimilar?

Biosimilar: A biologic shown to be "highly similar" to the reference biologic AND has "no clinically meaningful differences in safety and effectiveness" from the reference product.
Biosimilar Development Pathway

- No discovery (it already exists)
- Shortened clinical campaign
  - No Phase II
  - Some combine Phase I and Phase III
  - Only one Phase III necessary

Analytical is King

- Practical phase
  - Analytic
    - Biophysical
    - Structural
    - In vitro functional
  - Toxicology
- Clinical phase
  - PK
  - Efficacy
  - Safety

Directed Development in Biosimilars

- Highly iterative process
- Structure = Function

Biological Characteristics

- Binding Assays
- Functional Assays
  - Full-associated functions
    - Assays
Overview of Biologics Manufacturing Process

Thoughts on Industry

- Pros/Cons: Big or small company
- Optimize your resume! (please)
  - Cover letter necessary?
  - Know your resume
- Interview tips
- Questions?
Control Release Anesthetics to Enable an Integrated Anesthetic-MSC Therapeutic

Mollie Davis
October 28, 2016

Introduction

Local Anesthetics (LA):
- Minimize pain and discomfort
- Act directly on voltage-gated sodium channels and reversibly block the conductance in neurons

Common local anesthetics include bupivacaine, lidocaine, and ropivacaine

Mesenchymal Stromal Cells (MSCs):
- MSCs are an attractive option for tissue engineering and regenerative medicine applications because:
  - Multi-lineage differentiation potential
  - Immunomodulatory functions
  - Generally non-immunogenic


Problem

Effect of Local Anesthetics on MSCs:
- LAs affect the MSC:
  - Proliferation capacity
  - Differentiation potential
  - Adherence phenotype
  - Secretome
  - Immunomodulatory function
  - Viability

In a potency and time dependent manner

A cell therapy must be developed that can avoid compromising the integrity and potency of an MSC therapy and still deliver the necessary level of comfort to the patient.

Introduction/Objectives

Bupivacaine-loaded Liposomes
- A bilayer of lipids surrounding bupivacaine
- Bupivacaine slowly leaks through the bilayer

Hydrogel-Liposome Construct
- Liposomes are encapsulated in alginate hydrogel to further slow down the drug release.

Hydrogel-Liposome System
- Bupivacaine Diffusivity: 8.5E-15 mol/m3 in system, 1E-10 mol/m3 in media

Results-Previous Work

- Release from liposome alone within 24 hours
- Encapsulation of liposomes in alginate led to a sustained release
- Determined using LCMS

Previous studies demonstrate bolus dose of 0.1mM allowed for 90% cell viability

<table>
<thead>
<tr>
<th>Concentration</th>
<th>0mM</th>
<th>0.01mM</th>
<th>0.1mM</th>
<th>0.5mM</th>
<th>1mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Viability at 24 Hours</td>
<td>100±4%</td>
<td>95±4%</td>
<td>75±2%</td>
<td>64±0%</td>
<td>43±2%</td>
</tr>
<tr>
<td>% Viability at 48 Hours</td>
<td>100±4%</td>
<td>105±4%</td>
<td>90±2%</td>
<td>62±3%</td>
<td>38±4%</td>
</tr>
</tbody>
</table>

Alginate-liposome formulation should have a release profile that does not exceed 0.1mM at a given time to ensure high cell viability.

Results-Diffusivity

- Bupivacaine Diffusivity: 8.5E-15 mol/m3 in system, 1E-10 mol/m3 in media
- Construct ensures concentration remains low
Results - In Vitro Viability

<table>
<thead>
<tr>
<th>Bolus Dose</th>
<th>Liposomal Alginate Hydrogel Construct</th>
<th>Liposomal Alginate Hydrogel</th>
<th>Liposomal Alginate Hydrogel</th>
<th>Liposomal Alginate Hydrogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mM</td>
<td>1mM</td>
<td>1mM</td>
<td>1mM</td>
<td>1mM</td>
</tr>
</tbody>
</table>

Fl/Cell Number

MSC Viability at 96hrs

* Indicates significance <0.05    # indicates significance <0.0001

Results - In Vitro Functionality Testing

PGE2 Secretion

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1mM</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

IL-6 Secretion

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1mM</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* <0.05 Significant difference from media control
** <0.001 Significant difference from media control
+ <0.05 Significant difference from respective bolus dose
++ <0.001 Significant difference from respective bolus dose

Conclusions

• Construct enables:
  • Controlled long term release of bupivacaine to MSCs
  • Increased MSC viability compared to Bolus
  • Elevated IL6 and PGE2 secretions compared to Bolus and Media controls

This formulation provides multi-day pain-mitigation and can be co-administered with MSC therapies

Future Work

• Additional in vitro studies must be conducted
  • Inducing an inflammatory environment
  • Co-culture of MSCs with Macrophages
• Additional secretome analysis
  • Immunomodulatory molecules
  • Metabolic activity
• Model Analysis:
  • Cell uptake component
  • Gravity
  • Human geometry
• In vivo efficacy testing

Thank you

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• Biotechnology Training Program
• GAANN-Match Fellowship
Analysis of Tumor Growth and Anti-Tumor CD8+ T Cell Localization in Mice Concomitantly Challenged with Melanoma and Influenza

Jenna Newman
October 28, 2016
Advanced Topics in Biotechnology I

Immunotherapy: History and Present

Coley (1862-1936)

Coley’s Toxin: Deploying Bacteria to Combat Cancer

Dendritic Cell Vaccines

• PD-1 blockade
• CTLA-4 Blockade

Coley’s Toxin in the 21st Century

• The relationship between cancer and viruses is still under investigation

• Oncolytic viruses are used as cancer therapies (i.e. T-VEC)

• Infection with an oncogenic virus can lead to the development of cancer (i.e. HPV)

• QUESTION: How does infection with non-oncogenic viruses affect tumor development?

Coley’s Toxin in the 21st Century

Mice Concomitantly Challenged with Influenza and Melanoma Exhibit Faster Tumor Growth than Mice Challenged with Melanoma Alone

Anti-Tumor CD8+ T Cells Are Found at Higher Proportion in the Lung and a Lower Proportion in Tumor in Co-challenged Mice

Conflicting Data: Mice Co-Challenged with Melanoma and Influenza Exhibit Slower Tumor Growth
Gating for Ly6-C+ Gr-1+ Cells: MDSCs Observed at Decreased Frequency in Spleen of Flu/Mel Mouse

A Potential Explanation for Conflicting Data: Analysis of Experimental Timelines

Future Directions

- Plan experiments using a variety of B16-influenza timelines
- Repeat experiments using previously used B16 cell line
- Conduct plaque assays to more accurately determine PFU of administered virus
- Ensure that viral stock is not contaminated
- Inject influenza intratumorally; does it behave as an oncolytic virus?
- Combine with immunotherapy; what effects are observed?

Acknowledgements

- NIH-Rutgers-Biotechnology Training Grant T32GM008339-26
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Shengguo Li
Julia Lin
Saeed Tarabichi
Andrew Zloza
Industrial Job Search: Experiences and Lessons Learned

Mehdi Ghodbane
11-November-2016

Outline
• Disclaimer
• My Background
• Academic vs. Industrial Job Search
• My experience finding a job
• Lessons Learned
• Networking
• Timing
• Interviewing
• Summary and Advice
• Question/Answer

Disclaimer
ALL OF THE COMMENTS AND OPINIONS EXPRESSED IN THIS PRESENTATION ARE THE INDEPENDENT VIEWS OF THE PRESENTER

My Background
• BS, Bioengineering, Lehigh University
  – Biopharmaceutical Engineering Track, 2003-2007
• Project Engineer, Alpharma Pharmaceuticals
  – Small Molecules-2007-2009
• PhD, Biomedical Engineering, Rutgers University
  – Yarmush Lab, 2009-2014
  – Biotechnology Training Program Member 2009-Present!
• Investigator, GlaxoSmithKline
  – Biopharmaceutical Advanced Manufacturing Technology, R&D

Academic vs. Industrial Job Search
• Follow your heart!
• Academic
  – Many opportunities available for graduating PhDs
  – Peers and professors have extensive network in academia
  – Most likely will be able to find something relatively quickly in your field
• Industrial
  – Positions for PhDs are scarce, inherent in organizational structures
  – Depending on thesis research, may be difficult to find something directly relatable
  – Networking is the biggest challenge
  – Process is slow

My experience

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan 2014</td>
<td>Saw the light!!</td>
</tr>
<tr>
<td>Feb 2014</td>
<td>Resume Time</td>
</tr>
<tr>
<td>Apr 2014</td>
<td>Not looking hard</td>
</tr>
<tr>
<td>June 2014</td>
<td>Phone Interview</td>
</tr>
<tr>
<td>Aug 2014</td>
<td>On-Site Interview</td>
</tr>
<tr>
<td>Nov 2014</td>
<td>Defense</td>
</tr>
<tr>
<td>Dec 2014</td>
<td>Hiring Freeze Continuing</td>
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<tr>
<td></td>
<td>No News</td>
</tr>
<tr>
<td></td>
<td>Hiring Freeze Announced</td>
</tr>
<tr>
<td></td>
<td>Marc Interview</td>
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</table>
My experience - Continued

<table>
<thead>
<tr>
<th>Post-Doc/Looking Hard</th>
<th>GSK Contract</th>
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</thead>
<tbody>
<tr>
<td>Jan 2015</td>
<td>Dec 2015</td>
</tr>
<tr>
<td>Feb 2015</td>
<td>Jan 2016</td>
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<tr>
<td>Apr 2015</td>
<td>Feb 2016</td>
</tr>
<tr>
<td>May 2015</td>
<td>Mar 2016</td>
</tr>
</tbody>
</table>

My name is Mehdi Ghodbane, and I am finishing the 5th year of my PhD in Dr. Yarmush’s lab at Rutgers and am planning to defend my thesis by the end of 2014. I’m not sure if you remember, but we met [-----]. I am in the process of looking for industrial positions and I was hoping to network with you and learn more about [Company Name] and potential opportunities for someone with my background. I would greatly appreciate the opportunity to speak with you. I look forward to your response, thank you for your time.

Networking

**Opinion:** Applying to jobs online is highly unlikely to result in a phone screen
- Unless you are a great match, resume will most likely be overlooked
- A reference, on the other hand, is almost a guarantee that your resume will be read
- A phone screen is likely
- References are acquired through networking, something you should be doing no matter how far along you are in your PhD (and beyond)
  - Peers
  - Professors/industry guests
  - Peers of peers (LinkedIn)
  - Friends (even those outside of science)

- People are willing to have networking calls

Timing

**Critical piece to finding jobs, synonymous with luck**
- Someone must have a need with someone with your skill set at the same time you are searching for the opportunity
- Maximize your chances by networking as much as possible.

- Certain times throughout the year are better for hiring
  - End of year, headcount is approved
  - People come back from the holidays and take a few weeks to settle in
  - Groups eager to fill headcount and start the process in late Jan/Feb
  - November/February are very slow for hiring, mainly backfills only which may not be best for entry level PhDs
  - **REMEMBER, THE PEOPLE RECRUITING HAVE DAY JOBS**
  - Process can take several months (expect it)

Job Search Summary

- 2+ years from starting my resume to full time position
- 1 year to get contract job
- Was networking and applying to jobs constantly
- Need a little luck
- Not necessarily how it will work out for you
- Knew what I wanted to do, wasn’t a great match with my thesis research

- **Biggest Mistake/Regret:** Neglecting job search while finishing thesis
  - Initial networking was so successful, took my foot off the gas
  - Was worried I would get a job offer before being ready to defend

Networking Request

- A way to make a connection
  - Gain information about upcoming expansion/ jobs
  - Setup a potential reference
  - No pressure situation for the person you contact

My name is Mehdi Ghodbane, and I am finishing the 5th year of my PhD in Dr. Yarmush’s lab at Rutgers and am planning to defend my thesis by the end of 2014. I’m not sure if you remember, but we met [-----]. I am in the process of looking for industrial positions and I was hoping to network with you and learn more about [Company Name] and potential opportunities for someone with my background. I would greatly appreciate the opportunity to speak with you. I look forward to your response, thank you for your time.

- Be polite, if the person doesn’t respond, kindly follow up then forget it
- Nothing to lose

Interviewing

- You are being judged
- Expect to give a seminar
  - Main assessment of your scientific skills
  - Make it perfect, only part of an interview you can control
  - The way you present and answer questions says a lot about you

- Expect a rigorous process
  - All day interviews
  - Possible dinner

- Don’t be arrogant, show you are easy to work with
- **ASK QUESTIONS!!**
Summary/advice

- Always network
- Set up as many networking calls as possible, be shameless
- Start early, at least 1 year prior to defense
  - Never too early to start, and don't let up until you have an offer
- Expect the hiring process to take up to 4-6 months from your phone screen
- Timing is everything. Try to target early in the year
- Prepare for interviews, expect a rigorous process
- BE PERSISTENT
- YOU CAN DO IT!

Acknowledgments

- Mary Ellen Presa
  - >6 biotech presentations

Questions
Effects of NMDAR Agonists on the Expression and Function of NOS1AP, a Protein Encoded by a Schizophrenia Risk Gene

Kirsten Svane
November 11, 2016
Advisor: Dr. Bonnie Firestein

Background on Schizophrenia

- Etiology: combination of genetics and environment
- Symptoms: positive, negative, cognitive
- Problem: Current medications (antipsychotics, dopamine antagonists) fail to treat to negative and cognitive symptoms

Cellular features of schizophrenia

- Patients with schizophrenia show fewer dendrites and spines

What causes dendritic alterations in subjects with schizophrenia?

- NOS1AP – schizophrenia risk gene
  - Enhanced expression in DLPFC in individuals with schizophrenia
  - 2 isoforms studied: long and short
  - Decrease dendritic branching in embryonic rat hippocampal neurons

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Length</th>
<th>Effect of overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>501 amino acids</td>
<td>Decrease dendrites DIV 0-12</td>
</tr>
<tr>
<td>Short</td>
<td>211 amino acids</td>
<td>Decrease dendrites DIV 5-7</td>
</tr>
</tbody>
</table>

Role of NOS1AP (CAPON) in NMDA Receptor Functioning

Question: How do NMDAR agonists affect NOS1AP?

Goal 1: Can NMDAR agonists lower NOS1AP expression?

Goal 2: Can they correct dendrite branching abnormalities when NOS1AP is overexpressed?
**Goal 2: How do NMDAR agonists affect dendrite branching?**

- Transfect cDNA encoding NOS1AP isoforms DIV6 → Treat with drugs DIV7 → fix and immunostain for GFP and MAP2 DIV8

**DIV6:** Transfect cortical neurons to OE NOS1AP isoforms

**DIV7:** Treat with NMDAR agonists

**DIV8:** Immunostain, Image, and Sholl analysis of dendrites

---

**Overexpression of NOS1AP-Long and NOS1AP-Short significantly decrease dendrite branching in cortical neurons**

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFP</th>
<th>Glyx-13</th>
<th>D-Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO-GFP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyx-13-N-L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO-N-L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blue line * p<0.05 for N-L vs. DMSO
Orange line * p<0.05 for N-S vs. DMSO

3 replicates, N=40-48 neurons per condition
Two-way ANOVA followed by Bonferroni
All images taken at 20x
Error bars represent SEM

---

**Treatment with Glyx-13 restores dendrites near soma when NOS1AP-Long is overexpressed**

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFP</th>
<th>Glyx-13</th>
<th>D-Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO-GFP</td>
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<td></td>
</tr>
<tr>
<td>Glyx-13-N-L</td>
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<tr>
<td>DMSO-N-L</td>
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</tbody>
</table>

3 replicates, N=40-48
Two-way ANOVA followed by Bonferroni
All images taken at 20x
Error bars represent SEM

---

**Glyx-13 treatment has no effect on dendrites when NOS1AP-Short is overexpressed**

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFP</th>
<th>Glyx-13</th>
<th>D-Serine</th>
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<td>DMSO-N-S</td>
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<tr>
<td>Glyx-13-N-S</td>
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</table>

3 replicates, N=40-48 neurons per condition
Two-way ANOVA followed by Bonferroni
All images taken at 20x
Error bars represent SEM

---

**D-Serine treatment trends towards increasing distal dendrites when NOS1AP-Long is overexpressed**

<table>
<thead>
<tr>
<th>Condition</th>
<th>GFP</th>
<th>Glyx-13</th>
<th>D-Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO-GFP</td>
<td></td>
<td></td>
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<tr>
<td>DMSO-N-L</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D-Serine-N-L</td>
<td></td>
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</tbody>
</table>

3 replicates, N=40-48 neurons per condition
Two-way ANOVA followed by Bonferroni
All images taken at 20x
Error bars represent SEM
D-serine treatment has no effect on dendrites when NOS1AP-Short is overexpressed

Conclusions and Future directions

• Conclusions
  – Treatment with NMDAR agonists significantly reduces expression of NOS1AP isoforms
  – Treatment with NMDAR agonists reverses the decrease in dendrite branching that occurs when NOS1AP-Long is overexpressed
  – Possible treatment option for schizophrenia?

• Future and continuing work:
  • Do these findings hold true in vivo?
  • Inject rats with drugs → NOS1AP expression in cortex
  • Is NOS1AP expressed in cheek cells and is it elevated in patients with schizophrenia?
  • Biomarker for schizophrenia?

Acknowledgements

• Dr. Bonnie Firestein
• Committee members:
  – Dr. Jennifer Moore
  – Dr. Gabriella D’Arcangelo
  – Dr. Steven Silverstein
• Dr. Kristina Hernandez
• All members of Firestein Lab
• Ericka Asis
• Biotechnology Training Grant
• Neuroscience Graduate Program
Identifying factors involved in heterochromatin maintenance and epigenetic inheritance

What is epigenetics?
- Encompasses all sequence-independent processes that modulate gene expression

Why do we study epigenetics?
- Epigenetic dysregulation involved in many diseases
- Aberrant epigenetic state linked to cancer progression
- Implicated in cellular senescence & aging

Position Effect Variegation (PEV)
- Transcriptional silencing of a gene by heterochromatin spreading
- Stably inherited in subsequent generations

Screening for genes involved in centromeric silencing by transposable element-mediated mutagenesis

Successive selections enrich for genes involved in centromeric silencing
Successive selections enrich for genes involved in DNA replication, repair, and HR.

How are chromatin states inherited?

Proteomic analysis of replicating heterochromatin and euchromatin.

Establishing a human cell culture model of PEV.

Acknowledgements

- Dr. Mikel Zaratiegui
- The Zaratiegui lab
- NIH T32 GM008339/ Rutgers Biotechnology Training Program

Thank you!
Endogenous Neural Stem Cell Activation and Applications After Traumatic Brain Injury

Jeremy Anderson
Dr. Li Cai Lab
Department of Biomedical Engineering, Rutgers University
16:125:603 Topics in Advanced Biotech I
9 December 2016

Adult Neurogenesis

Notch regulates production of neural progenitor cells and neuroblasts

Adult neurogenesis occurs in
- Subgranular zone (SGZ) of dentate gyrus in the hippocampus
- Subventricular zone (SVZ) of the neocortex

Increased neurogenic response in SVZ of DG post-TBI (Villapol, 2013)

New neurons integrate into circuitry
- Involved in learning, memory, and motor functions

Genes driving NSC activation post-TBI are not fully defined

Understanding NSC activation may identify new targets to enhance recovery post-TBI and develop novel therapies

Overview

Human TBI
- 85-89% from blunt, closed head trauma (Flierl, 2009)
- Penetrating injuries in 0.8-3% of patients (Flierl, 2009)

TBI induced by weight-drop device
- Replicates biomechanics/pathobiology of injury
- Notch1Cre2-GFP transgenic mice (8-12 weeks)
- Skull is exposed
- 3-bcm free falling guided weight (327g)
- Focal blunt injury
- Results in neuroinflammatory response (Flierl, 2009)

Research Question & Hypothesis

- Research Questions: What cellular and transcriptome changes are induced by TBI, and can this neurogenic response be promoted post-TBI?
- Hypothesis: Endogenous NSCs respond to TBI and have the potential to recover TBI-induced cell damage.
- Significance & Innovation:
  - Understand genes responsible for the neurogenic response post-TBI
  - Using single-cell transcriptome analysis, identifying novel genes driving neurogenesis will identify pathways associated with TBI

Closed Head Injury (CHI) Model

Research Goal: Identify and characterize the activation of endogenous NSCs after TBI and determine their potential in injury repair and neural regeneration

Aim 1: Develop a CHI model to study neurogenesis post-CH (post-TBI)
Aim 2: Characterize GFP+ NSC identity, activation, and fate post-TBI
Aim 3: Identify genes (and pathways) activated after TBI which drive NSC activation/injury response
Aim 4: Validate genes identified activated after injury in adult/mice CH further characterize TBI model
**Rutgers**

**GFP+ NSCs Increased Post-CHI**

GFP, DAPI

Control, male

Injured, 2 dpi, male

CHI induces significant NSC activation in the hippocampus (GFP, DAPI)

**GFP+ NSCs express NSC/NPC markers**

Nestin, GFP

Control, female

Nestin, GFP

Control, male

DCX, GFP

Control, female

NeuN, GFP

Injured, 2 dpi, male

Injured, 2dpi, male

Injured, 2dpi, male

GFP+ activated cells express Nestin, DCX, and NeuN indicating GFP+ cells are NSCs and neurons

**Injury markers identified after injury**

![Graph showing injury markers](image)

Identification of brain injury/inflammatory markers after injury using qPCR prior to single-cell RNA-seq analysis

**3: Identifying brain injury markers**

<table>
<thead>
<tr>
<th></th>
<th>3 hr after injury (fold change increased)</th>
<th>48 hr after injury (fold change increased)</th>
<th>72 hr after injury (fold change increased)</th>
<th>Conclusion</th>
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</thead>
<tbody>
<tr>
<td>ApoE</td>
<td>-0.5</td>
<td>-1.1, -0.3</td>
<td>-1.0, -1.2</td>
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<td></td>
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<td>-1.0, -0.7</td>
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<tr>
<td>Tau</td>
<td>0.8</td>
<td>1.0, 0.4</td>
<td>0.8, 0.9</td>
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<tr>
<td></td>
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<td>0.4, 0.6</td>
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<td>IL-1alpha</td>
<td>0.3</td>
<td>0.7, 0.2</td>
<td>0.8</td>
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<tr>
<td>IL-1beta</td>
<td>0.0</td>
<td>0.9, 0.2</td>
<td>1.8, 1.2</td>
<td>*Higher in injured 3 days after injury</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0, 0.6</td>
<td></td>
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<tr>
<td>GFP</td>
<td>0.8</td>
<td>1.3, 1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identification of brain injury markers after injury using qPCR prior to single-cell RNA-seq analysis

**Future Work:**

- Further characterize/adjust model severity using qPCR quantification of brain injury markers
- Investigate single-cell transcriptome active after injury using single-cell RNA-sequencing
- Validate identified genes in vitro and/or in vivo

**Research Summary**

**Elucidate cellular and transcriptome changes induced by TBI, and determine their potential in injury repair and neural regeneration**

**Acknowledgements**

- NIH Biotechnology Training Program: T32GM008339
- NJSCR
- PI: Dr. Cai
- Cai Lab Members

**Thank You!**
NanoScript: A Nanoparticle-based Artificial Transcription Factor For Gene Regulation And Stem Cell Differentiation

December 9th, 2016
Christopher Rathnam

NanoScript Replicates TF Proteins
Objective: Develop a nanoparticle-based platform called NanoScript to replicate the multi-domain structure and gene-regulating function of TFs

NanoScript Components:
- NLS Peptide – facilitates nuclear targeting
- DNA Binding Domain – binds to specific DNA sequences
- Activation Domain – initiates transcriptional activity
- Linker Domain (LD) – tethers multi-domain components together

NanoScript is Stable in Physiological Environments
- The stability of NanoScript was tested in various physiological solutions for 3 days
  1. NanoScript in water is stable
  2. NanoScript in phosphate buffered saline (PBS) is stable
  3. NanoScript in cell culture media containing 10% serum is stable

Modify STFs for Efficient Gene Activation
Objective: Develop a versatile, highly efficient gene regulating nano-platform for activating targeted genes in a non-viral manner.

Limitation of STFs
1. Low synthesis efficiency
2. Poor membrane permeation
3. Lack of nuclear targeting moiety
4. Low gene activation limited to a 1:1 domain ratio

Nanoparticle-based STF
1. Separate synthesis
2. Efficient membrane permeation
3. Attachment of nuclear targeting moiety
4. Domain ratios are tunable

Construction of NanoScript
- NanoScript construction is a two-step process
  1. synthesize PEG-terminal biomolecules → enhanced stability + AuNP conjugation
  2. Functionalize AuNPs to create the NanoScript platform

Synthetic Transcription Factors
- Synthetic analogues of natural TFs
  - Comprised of both domains
- DNA Binding Domain (DBD) is most critical:
  - Must be very specific for targeted genes
  - High binding affinity to DNA is essential
- Hairpin polyamides are one effective small-molecule DBDs
  - comprised of N-methylpyrrole and N-methylimidazole
  - Sequence rearrangement to complement almost any gene → vast potential for therapeutic implications
Hearing Loss

- There are many reasons for hearing loss:
  - Exposure to loud noises
  - Direct injury to the ear
  - Birth defects
  - Exposure to ototoxic drugs

- Hearing aids amplify sound and do not usually help when there is death of hair cells or neurons in the ear.

Current techniques to treat sensory neural hearing loss include cochlear implants

- Directly convert sound via a microphone into electrical stimulation of the auditory nerves.

- Risks:
  - Tinnitus.
  - Vertigo or dizziness.
  - A skin wound infection.
  - Numbness in the area around the person's ear.
  - Blood or fluid collection at the surgery location.
  - Unforeseen complications due to the surgical procedure.

Transcription factors for Hair Cell development

- Gfi1, Pou4f3, Atoh1 (GPA)
  - Gfi1 and Atoh1 are involved in hair cell differentiation
  - Pou4f3 is involved in hair bundle formation
  - Thought to be enough to transdifferentiate supporting cells into sensory hair cells

Immortalized Otic Progenitor cells

- Mouse otic progenitor cells that were immortalized for the study of otic neurogenesis and hair cell development

NanoScript Nuclear Localization

- For gene activation, the NanoScript must:
  - reach the nucleus
  - remain intact

- (B,C) overlap between the nucleus (red) and NanoScript (green)

- (D) TEM image shows NanoScript (black dots) inside the nucleus

GPA NanoScript

- Treatment of GPA NanoScript increases Myosin 7a RNA, a marker for hair cell differentiation

- NanoScript is able to enter cells and cause little cytotoxicity
ASCL1 NanoScript

- Dll1 a direct target of ASCL1 was upregulated with the treatment of NanoScript
- Neurons were able to show more mature morphology and protein expression

Future Plans

- Test time dependent delivery of NanoScript for hair cell differentiation
- Quantify nuclear vs cytoplasmic localization of NanoScript
- Test different ratios of domains on NanoScript for optimal effect

Acknowledgements

- PI: Dr. KiBum Lee
- Funding: Biotechnology Training Fellowship NIH T32 GM008339

Questions?

Supplementary Slides

TFO vs Polyamide in IPSC-NSC
Synthetic TFs Activate Gene Expression

- STFs are effective small molecules for regulating gene expression

STFs are increasing nuclear extract (nuclear extract) (live cells) Increasing demand.

Journal of the American Chemical Society 2004, 126, 15940.

Challenge: Need to modify STFs for live cell delivery with enhanced activation.

Hairpin Polyamide Design

- The hairpin polyamides structure acts as the DNA-Binding Domain (DBD)
  - Bind to target DNA sequences
  - Pyrrole bind to A-T base pairs on DNA
  - Imidazole binds to G-C base pairs on DNA
  - High binding affinity that is comparable to naturally TF proteins

Hairpin Polyamide Design

Three Components of NanoScript

- Nuclear Localization Signal Peptide (NLS)
- Transactivation peptide (AD)
- Hairpin polyamide (DBD)

NLS (facilitates nuclear targeting and localization)
AD (Recruits proteins to initiate transcription)
DBD (Binds to targeted DNA sequence)

NLS: 68.2 ± 1.0%
AD: 22.8 ± 2.6%
DBD: 9.0 ± 2.1%

The ratios of the three components on NanoScript surface were specifically chosen.

NLS increased for enhanced nuclear uptake.
AD increased for strong transcriptional initiation.
DBD has high DNA binding affinity.

Characterization of Biomolecules on NanoScript

- The ratios of the three components on NanoScript surface were specifically chosen.
- Characterized using HPLC

Approximate Peptide Ratio
- NLS: 68.2 ± 1.0%
- AD: 22.8 ± 2.6%
- DBD: 9.0 ± 2.1%

Gene profile of developing cochlea

- Scheffer et. al. Journal of Neuroscience 2015
- Stem cell differentiation and reprogramming is a highly pursued field of research.
  - Enormous clinical potential
  - Patient specific treatment
  - Generation of almost any desired cell type

Stem Cell Differentiation

- Guiding stem cell differentiation is all about Transcription Factors
  - iPS cells (Oct4 and Sox2)
  - Source Cells (drk, A22, and 6949)
  - Chorogonital (Avd)
  - Muscle cells (MyoD and Myogenin)

Transcription Factors Regulates Differentiation

Characterization of Biomolecules on NanoScript

Gene profile of developing cochlea

NLS: 68%
AD: 9%
DBD: 23%

Characterization of Biomolecules on NanoScript

Gene profile of developing cochlea

NLS: 68%
AD: 9%
DBD: 23%
16:125:604 Topics in Advanced Biotechnology  
Spring 2017  
Fridays, 9:00-11:00, BME Room 122

- 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.
Reversing the Aging Process

Martin Yarmush, MD, PhD
Department of Biomedical Engineering
Rutgers University

16:125:604: Topics in Advanced Biotechnology II
Friday, January 20, 2017

Definitions

- **Lifespan** describes how long an individual lives.
- **Life Expectancy** is a population-based estimate of expected duration of life for individuals at any age, based on a statistical ‘life table’.
- **Maximum lifespan** is the age reached by the longest-lived member of a species.
- “**Healthspan**”

Life Expectancy: Scientific Advances

<table>
<thead>
<tr>
<th>Year</th>
<th>Life expectancy at birth</th>
<th>Life expectancy at age 65</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>1900</td>
<td>47.9</td>
<td>50.9</td>
</tr>
<tr>
<td>1930</td>
<td>58.0</td>
<td>61.3</td>
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<td>1950</td>
<td>65.6</td>
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<td>1970</td>
<td>67.1</td>
<td>74.9</td>
</tr>
<tr>
<td>1980</td>
<td>69.9</td>
<td>77.5</td>
</tr>
<tr>
<td>1990</td>
<td>71.4</td>
<td>78.3</td>
</tr>
</tbody>
</table>

Increase 23.5 27.4 3.6 6.8
According to data compiled by the Social Security Administration:

- A man reaching age 65 today can expect to live, on average, until age 84.3
- A woman turning age 65 today can expect to live, on average, until age 86.6
- About one out of every four 65-year-olds today will live past age 90, and one out of 10 will live past age 95

Why should we care?

Populations are Getting Older

The number of people aged 60 years or older will rise from 900 million to 2 billion between 2015 and 2050 (moving from 12% to 22% of the total global population).

Population aging is happening much more quickly than in the past

Time for percentage of population older than age 60 to double

France took 150 years to adapt to a change from 10% to 20% in the proportion of the population that was older than 60 years; places such as Brazil, China and India will have slightly more than 20 years to make the same adaptation. Two key drivers 1) falling fertility rates, 2) people living longer.

The Aging Population

As The Baby Boom Generation enter years of 65+ in 2011 there will be an estimated 74% growth in population 65 – 74 by the year 2020. This growth is exceeding the under 65 population which only sees a growth of 24%
Normal Effects of Aging on the Body

Brain/Mind

Hematopoietic

Body

Blood

Hematopoietic

Red cell life span is unchanged
Neutrophil levels remain constant
Platelets unchanged
Lymphocyte levels decline
Age 75 compared to age 30:
92% of brain weight
84% of basal metabolism
70% kidney filtration rate
43% of maximal breathing capacity

Theories of Aging
- Programmed Aging Theory
  - Hayflick Phenomenon
  - Telomere shortening
- Somatic Mutation Theory
- Metabolic Theory/Waste Accumulation
- Wear & Tear Theory
- Cross-linking Theory
- Free Radical Theory
- Neuroendocrine
- Immunological

The Hayflick Limit (1961)
Pre-1961: “All metazoan cells are potentially immortal. Ageing not cell autonomous”

Hayflick and Moorhead (1961) Exp Cell Res

1. Isolate fibroblasts from human skin
2. Place in culture with medium
3. Cells divide and form confluent layer
4. Discard half the cells, allow remainder to grow to confluency = one passage
5. Continue to passage the cells
6. Cell replication slows and stops after 50 ± 10 passages: cells have reached the Hayflick limit and undergone replicative senescence

Successful or Healthy Aging
Chronologic age and physiologic age not the same
Due to complex interactions of genetics and environment
Individuals “age” at different rates and there is significant variability
Successful or Healthy Aging
Prevalence of disease increases with age
Pathways of aging:
- Aging with disease and disability
- Usual aging; absence of pathology but presence of decline in function
- Healthy aging; no pathology or functional loss

Healthy Aging is an Investment, not a Cost

Jackson Labs reports that "only diet restriction and the panhypopituitary dwarf mutation have been shown to delay senescence and increase life span on a variety of long-lived genetic backgrounds in experiments replicated by independent laboratories. In unrelated genotypes that express diverse diseases of aging, these treatments delay expression of multiple biomarkers of aging and increase life span".

Evidence for a limit to human lifespan
Xiao Dong, Brandon Milholland, & Jan Vijg
Nature 538, 257–259 (October 13, 2016)

What about lifespan?
Some conserved pathways and aging interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Yeast</th>
<th>Women</th>
<th>Mouse</th>
<th>Mice</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td></td>
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<td></td>
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<tr>
<td>Sirtuin</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Ocampo et al.</td>
<td></td>
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</tr>
</tbody>
</table>

In the 1950s, Clive M. McCay et al. at Cornell were the first to apply parabiosis—a 150-year-old surgical technique—to the study of aging. Parabiosis involves connecting two animals together surgically, mimicking the natural occurrence of conjoined twins, allowing the opportunity to test how circulating factors in the blood of one animal behave when they enter another. When McCay did necropsies he found the cartilage of the old rats appeared youthful, as if it had been rejuvenated by the exposure to young blood. Experiments at Stanford in the early 2000s supported the theory that there are compounds in the blood of the young mice that could stimulate old stem cells and revitalize aging tissue. In 2008, scientists at the UC Berkeley (formerly of the Stanford team) identified one of the anti-aging factors circulating in the blood as oxytocin. When injected systemically into older mice, oxytocin quickly (in weeks) regenerated muscles by activating muscle stem cells. Other blood components have stood out: GDF11 (controversial), and a second factor known as B2μ, which peaks in the blood of old mice and humans.
Life Expectancy Gains for Modern Eunuchs

<table>
<thead>
<tr>
<th>Age at castration</th>
<th>Increase in Life Expectancy of Modern Eunuchs</th>
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<tbody>
<tr>
<td>11</td>
<td>7.0</td>
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<tr>
<td>13</td>
<td>4.8</td>
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<td>15</td>
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<tr>
<td>27</td>
<td>1.8</td>
</tr>
<tr>
<td>29</td>
<td>1.7</td>
</tr>
</tbody>
</table>

“You don’t stop laughing when you grow old, you grow old when you stop laughing.”
— George Bernard Shaw
Rejuvenation of Aged Progenitor Cells by Exposure to a Young Systemic Environment

Irina M. Conboy, Michael J. Conboy, Amy J. Wagers, Eric R. Girma, Irving L. Weissman, and Thomas A. Rando

Mollie Davis
Biotech Presentation
January 20, 2017

Background

• Ageing
  – Decline of tissue and organ function
  – Genetic and mitochondrial dysfunction
  – Reduced tissue regeneration
  • Notch Pathway

“Aged muscle successfully regenerates when grafted into muscle in a young host”

Systemic factors support regeneration in old animals and modulate key molecular pathways that control regeneration.

Implication: Old tissue might be made to regenerate \(\rightarrow\) rejuvenation from old state to young state.

Notch Signaling Pathway

• Cell-Cell Interaction
  – Transmembrane Protein
  – Extracellular binds Delta
  – Intracellular cascade to nucleus \(\rightarrow\) gene expression
  – Different Notch activation leads to different gene expression
  • Involved in embryogenesis and regeneration

Method

Parabiotic Pairings

A simple surgery

In Vivo Surgery Timeline

T=0days
Parabiosis Surgery
Muscle Injury
BrdU Injection
T=37days
BrdU Injection
Muscle, Liver Analyzed
T=39days
T=40days
Results

Cultured Overnight in Serum w/wo myofibril explants

Cells isolated from mice

Conclusion/Discussion

• Systemic factors
  – Modulate molecular signaling pathways
  – Young animal environment promotes regeneration
  – Old animal environment either inhibits or fails to promote regeneration
  – Can reverse decline of tissue regeneration

• Young systemic environment restores a younger profile of molecular signaling to aged progenitor cells in muscle and liver

Critiques

• Only analyze soluble factors
  – Disregards organ sharing, physical movement hindrances, etc.
  – Interested in drug to help with rejuvenation

• Controls?
  – Mouse alone with no parabiotic pairings
    • Only seen for liver BrdU studies
    – Sham surgery control for inflammation

• Functionality Testing?
• Dose response? Arbitrary Time Point?
Heterochronic Blood Exchange

Ryan Guasp  
Advanced Topics in Biotechnology II  
January 20, 2017


Heterochronic Parabiosis

Findings
- Enhances myogenesis, hepatogenesis, neurogenesis, osteogenesis, and overall cognition in aged mice
- Inhibits stem cells in young mice exposed to old blood

Caveats
- Continuous access to youthful organs
- Environmental enrichment from young animal
- Exercise
- Pheromones
- Circulating cell exchange (including stem cells)
- No identified blood factors

A new protocol: heterochronic blood exchange

- Small, motorized device using a computer-controlled peristaltic pump
- Exchanges 10% of animals total blood (150 μL) at a time
- 15 blood exchanges of 150 μL, two times within a 24 hour period
- Young: 3 months  
Old: 23 months

Heterochronic blood exchange improves muscle regeneration in old mice

Old blood may reduce performance in young mice
Old Blood decreases neurogenesis in young mice

Dentate Gyrus of the Hippocampus

Age of mouse | Age of blood
Injured Uninjured

Young blood improves hepatogenesis in old mice

Liver of old mice exchanged with old blood

Age of mouse | Age of blood
Injured Uninjured

B2M, a marker of aging, is modulated by heterochronic blood transfer

Summary

• Young blood improves muscle injury healing in older mice
  – New and thicker muscle fiber growth
  – Reduced fibrosis

• Old blood inhibits neurogenesis in young mice
  – This effect is magnified by muscle injury
  – Young blood has no detectable effect on neurogenesis in old mice

• Hepatogenesis is increased by young blood and response to injury
  – Muscle injury caused enhanced hepatogenesis in all samples
  – Young blood increased cell proliferation
  – Young blood decreased liver scarring and adiposity
  – Old blood decreases proliferation in young mice

Practical Consequences

• Young blood donors may be especially important for transfusions after injuries that damage muscle

• Donors beyond a certain age might be excluded, or marked specifically for isochronic recipients due to potential negative health outcomes.

• Systemic factors in the blood may facilitate muscle repair

• Systemic factors in the blood may inhibit neural stem cells and might be cleared by novel dialysis technique

• Heterochronic blood transfer may yield more therapies sooner than heterochronic parabiosis

Thank you to:

• Dr. Yarmush
• Ileana Marrero Berrios
• Tom Linz
• Kate O'Neill
• Mollie Davis

Questions?
Discussion questions

• What do you think were the main goals of these projects? Did the authors do adequate work to achieve these goals? What else needs to/could have been done?

• Are you willing to move forward/take action based on this work? Are you convinced? What should or should not happen next?

Discussion questions

• Do you have ethical concerns regarding this type of research?
• Is ageing a disease to be cured or is it part of the natural progression of life?
BioInks for 3D Printing and Free Form Fabrication of Regenerative Scaffolds

Faculty Presenter: David I. Shreiber, Professor, Biomedical Engineering
Student Presenters: Zachary Fritz, BME; Christopher Rathnam, CCB
Discussion Facilitators: Daniel Browe, BME; William Pfaff, BME
Student Coordinator: Madison Godesky, BME

Traditional, Subtractive Manufacturing
• Great for mass production of same part over and over again
• Different machines for different operations (drills, milling machines, grinders, …)
• Can be automated (CNC machines) or manual or both
• Series of operations
• Precision is key – build fixtures to ensure each piece is loaded in exactly the same way

You know what you want to make (exactly), and you make it over and over and over again.
But what if you aren’t sure?

3D-Printing, Rapid Protoyping, & Free-Form Fabrication
• “Additive Manufacturing”
• Rapidly test designs
• Accelerate concept-to-design-to-product
• “One-of-a-kind” designs
• Uses in education, manufacturing, food, …
• Tissue engineering & regenerative medicine

3D-Printing in Biomedicine
• Make patient-specific devices
• Control material orientation and porosity
• “Internal” and “External” features
• Combine multiple materials with spatial control
• Both synthetic and biologic materials
• 3D printing used for a wide range of applications:
  • Craniofacial implants, dental molds, crowns and implants, prosthetic parts, on-demand medical equipment, surgical models, scaffolds for tissue regeneration such as skin and bone, organ printing, and tissue models for drug discovery

General 3D Printing Process

3D-printing Approaches

- Fused Deposition Modeling (FDM)
- Direct ink Writing (DIW)
- Inkjet Printing
- Selective Laser Sintering (SLS)
- Stereolithography (SLA)
BioInks – Design Criteria

- Implantable biomaterials have specific requirements based on both physiological conditions and interactions with the body that make development as a bioink much more difficult than a general, printable material
  - Application/approach specific
  - Potential to print cells

- The bioink must:
  1. Be amenable to printing with one or more approach
  2. Biocompatible
  3. Have appropriate mechanical properties, both for structural integrity and function
  4. Have good degradation kinetics
  5. Form safe degradation byproducts
  6. Mimic tissue form and functions

- May be difficult to capture all of these criteria with one bioink

- Ultimately, a balance between all these parameters must be struck to develop a viable bioink

- Also must be concerned with resolution, reproducibility, quality control, scalability, cost, and regulatory hurdles

Plethora of potential, printable materials

Natural or synthetic (or both)?

Cells or no cells?

Printing approach(es)?

ECM-Derived Inks

- Few success stories that develop collagen as a bioink

Why did I pick this topic?

- Type I collagen
  - Ubiquitous protein that provides structural integrity
  - Also exerts significant influences on cell proliferation, differentiation, migration, gene expression
  - Mechanical stability, cytocompatibility, and biodegradability make it very desirable for medical applications, including tissue engineering
  - Many companies with collagen-based products: Integra Life Sciences, KCI/LifeCell, Organogenesis (Novartis), Collagen Matrix, Johnson & Johnson, Smith & Nephew, TEI Biosciences, Wound Care Innovations
  - Reactive lysine residues provide opportunity for biointerface control

Type-I Collagen as a Bio-ink

Difficult to use type I collagen for manufacturing customizing scaffolds or use as a bio-ink

- Hierarchical assembly into fibers is key for structural and functional properties
- Assembly is pH sensitive and temperature-driven
- Weak mechanical properties
  - Shear thinning
- Sticky
  - Few successful approaches
  - Complicated means of 3D printing
  - Must be additive

Key Issues

- Hierarchical assembly into fibers is key for structural and functional properties
- Assembly is pH sensitive and temperature-driven
- Weak mechanical properties
  - Shear thinning
- Sticky
  - Few successful approaches
  - Complicated means of 3D printing
  - Must be additive

Collagen Methacrylamide (CMA)

- Photocrosslinkable Collagen that retains ability to self-assemble but also allows for the spatiotemporal control of bioactive and mechanical properties
CMA Characterization

Tunable stiffness

Resistance to enzymatic degradation

Mechanical Degradation Protein Liberation

Good viability of encapsulated cells

Potential to photopattern stiffness and acrylated ligands

Serendipitous Discovery

CMA is Thermoreversible!

UV on/off

Drzewiecki et al, Langmuir, 2014

CMA is Thermoreversible…

But Photocrosslinked CMA is NOT Thermoreversible!

Free-Form Fabrication of CMA

Unassembled Gel

Cold-melted Gel

Assembled Gel

UV CrossLinking of Assembled Gel

Printing of Hydrogels and Scaffolds

Photomask

Hydrogel

Lyophilized Scaffold

Relyndran Scaffold

Cell Viability in Cell-Encapsulated Hydrogels

Results

- ~75% viability after 24 hrs
- Good pattern fidelity immediately after cold-melting
- Cellular compaction of CMA matrix demonstrates functional interactions between cells and CMA

- Improved pattern fidelity with larger photomasks
- Ability to make customised hydrogels and freeze-dried scaffolds

Scale bar = 5 mm, p < 0.05
What makes a good bioink?

- The bioink must:
  1. Be amenable to printing with one or more approaches
  2. Be biocompatible
  3. Have appropriate mechanical properties, both for structural integrity and function
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Subcutaneous Implant Model - Biocompatibility

Top: One week post-implant
Bot: Six weeks post-implant

RAT COLLAGEN
BOVINE COLLAGEN

DAPI

---

Shameless Self-Promotion

Presented by Zach Fritz

Presented by Chris Rathnam

---

800-883-8220

---

Keep in mind

---

Today.....
3-D Bioprinting

Feb 3, 2017

3-D Bioprinting

• The use of additive manufacturing for the generation of three-dimensional structures using biocompatible materials, cells, and supporting components to create living tissue-like complexes

• Used in
  – Transplantation
  – Disease modeling
  – Drug Testing

Scaffold-Free Bioprinting

• 3D printing without the use of scaffold material to hold the cells in place

• Advantages
  – No “foreign material” for transplantation
  – High initial cell density
  – High production of ECM
  – Greater number cell-to-cell interactions

• Limitations
  – Scale up is usually limited
  – Time for cells to form tight network
  – Usually need molds to hold cells in place

Three-dimensional bioprinting using self-assembling scalable scaffold-free “tissue strands” as a new bioink

Vin Yu, Kastin M. Meruelo, Jiongzheng Li, Weijun Peng, Iris Rivers, James A. Martin & Sandi P. Oates [2014]
Tissue Strand Synthesis

- Cells are seeded inside the microcapsule using a microsyringe
- Maintained a viability of 75%
- Strands of 8cm were fabricated for bioprinting

Mechanical Properties of Strands

- Measured over three weeks of culture
- Ultimate strength: 283.1 ± 70.36 kPa to 3371 ± 465 kPa
- Young's modulus: 1050 ± 248.6 kPa to 5316 ± 487.8 kPa
- Failure strain: 62.93 ± 12.83 to 91.46 ± 3.852

Characterization of Tissue Strands

- Strong proteoglycan deposition was shown after two weeks
- DMMB assay showed glycosaminoglycan levels in strand are comparable to native cartilage
- Gene expression was also quantified compared to monolayer cultured bovine chondrocytes

3-D Printing of "Tissue Strands"

Critiques

- Not really scalable
- Will it work with other cell lines?
- Limited "fusion" based on histology staining
- Limited integration of the transplant
- For the amount of effort needed does it actually perform any better?
3D Bioprinting Using a Templated Porous Bioink

James P.K Armstrong, Madeline Burke, Benjamin M. Carter, Sean A. Davis, Adam W. Perriman

**Background**
- 3D printing tissues and organs is intriguing, but challenging and not currently feasible for scaled up production
- One bioprinting dilemma: scaffolds or scaffold-free?
- Most scaffold-based bioprinting uses extrusion printing
- Selecting the ideal bioink is not trivial…

**Bioink Materials**
- Pluronic copolymers: non-ionic surfactants
  - Pros: undergoes sol-gel transition upon heating (micellar crystallization)
  - Cons: quickly degrades after cooling or in aqueous environment
- Alginate: naturally derived (brown algae) polysaccharide
  - Pros: crosslinking with addition of divalent salts, withstands aqueous environment
  - Cons: bad for extrusion printing—limited shear thinning capacity and interlayer adhesion, limited scale-up applications

**Hypothesis and Objectives**
- Both of these materials alone are poor bioinks…
- But what if we combine them?
- The hybrid material should:
  - Have properties amenable to extrusion printing
  - Maintain its structural integrity after deposition and in physiological conditions
  - Support cell growth, proliferation, and tissue differentiation

**Formulation Optimization**
- Varied concentrations of:
  - Pluronic F127 (11, 13, and 15 wt%)
  - Alginate (2, 4, and 6 wt%)
  - Later, medium CaCl$_2$ (1, 3, 5, 10 mM)
- Extrusion printed 10 mm x 10 mm x 2.4 mm rectangular prisms onto heated stage (for sol-gel transition)
- Crosslinked in 100 mM CaCl$_2$
- Incubated in cell culture medium for up to 5 days

**Formulation Optimization Results**
- Optimum: 13 wt% F127 and 6 wt% alginate
- F127 conc. affected printed structure
- Alginate conc. affected degradation rate
- Noticed after 5 days degradation due to Ca$^{2+}$ efflux
  - Solution: add 5 mM CaCl$_2$ to medium
Microstructure Characterization

- Used Fourier transform infrared spectroscopy to confirm that F127 is expelled from gel during washing and crosslinking steps.
- Used SEM to show:
  - F127 causes pore formation; ideal for nutrient transport, elasticity, ECM deposition.
  - Unidirectional calcium diffusion into causes alginate channels to be aligned anisotropically; ideal for guiding cells, nutrient transport.

Rheology and Compression Testing

- Used a rheometer for oscillatory testing to find:
  - Linear Viscoelastic Region (LVE): constant rate of stress increase wrt to strain increase.
  - Storage modulus (G') related to elasticity.
  - Loss modulus (G'') related to viscosity.
  - Flow index (n): exponent in power-law fluid relationship, indicates degree of shear-thinning.
- Used an instron for compression testing to determine Young’s modulus (E).

Rheology and Compression Testing Results

- Uncrosslinked gel is shear thinning (n = 0.126 ± 0.001), much better for extrusion than alginate alone.
- Viscoelastic, order of magnitude larger LVE.
- Crosslinked gel had a 50% higher Young’s modulus (E = 45 ± 4 kPa) than alginate.
  - Closer to soft tissue, e.g. hyaline cartilage (E = 79 ± 39 kPa).

Anatomical Structures

- Printed:
  - Tracheal cartilage ring
  - Ear
  - Nose (17.2 mm high).
- Change resolution by changing extrusion tip inner diameter.
  - Mean line width down to 0.19 ± 0.01 mm.

Cell Viability and Differentiation Studies

- Used human mesenchymal stem cells for all experiments.
- Added 3 x 10^6 hMSCs/mL to gel and over 5-10 days after printing looked at:
  - Extrusion efficacy.
  - Structural integrity.
  - Cell viability with live/dead staining.
- Final experiment: Double the cell density, added growth factors for chondrogenesis or osteogenesis to system, observed differentiation and ECM deposition with histological stains during 3-5 week period.

Cell Studies Results

- No observable effect on extrusion efficiency, structural retention.
- Cell viability remained high (83% @ 7 days) for duration of experiment.
- Cells differentiated with growth factors, deposited appropriate ECM:
  - Glycosaminoglycan and collagen for chondrocytes.
  - Inorganic calcium and phosphate for osteoblasts.
- Made an engineered tracheal ring with bioink, hMSCs, chondrogenic growth factors.
**Conclusions and Critique**

- Researcher’s conclusion: the hybrid bioink is greater than the sum of its parts
- But...
  - No in vivo testing (intended for tissue engineering/implantation)
  - Scalability might still be an issue (how deep will alginate cross-linking occur?)
  - Greater porosity doesn’t necessarily indicate strong in vivo performance
  - Chance of residual F127 being left behind?

**Acknowledgements**

- My team (Madison, Dr. Shreiber, Daniel, William, Chris)
- The Biotechnology Training Program
Personalized Medicine
Something old, something new
and a systems view

Ioannis (Yannis) P. Androulakis
Biomedical Engineering and
Chemical & Biochemical Engineering, Rutgers University
Department of Surgery, Rutgers-RWJ Medical School

In case I loose you

What I would like to discuss is: should we personalize the treatment or the patient individual?

Leading causes of death in the USA in the early 1900’s

Communicable diseases spread from one person to another or from an animal to a person. The spread often happens via airborne viruses or bacteria, but also through blood or other bodily fluid. The terms infectious and contagious are also used to describe communicable disease.

Infectious diseases and the germ theory of disease

“bad air from swampy areas” (Hippocrates, 460 BC)
The germ theory is a fundamental tenet of medicine stating that microorganisms invade the body and cause certain diseases. One must be able to isolate the microbe and either keep it away, or eliminate it, from the patient.

Samuelweis hypothesized that the trainee doctors were exposed to ‘cadaverous particles’ in the course of the autopsies they conducted, which they then transferred to the new mothers […] Samueléis proposed a connection between touching cadavers and a risk of infection […] He instituted a clinic-wide policy of mandatory hand washing between cutting up a body and assisting in a birth […] introducing a chlorinated lime solution to the hospital. In the first three months, death rates plummeted from one in ten to one in a hundred.

The germ theory: a fundamental concept

(I think) It defined the approach to disease treatment
Reductionist approach aimed at isolating cause/effect relations: Target the cause to eliminate the effect (symptom)
(most) Drugs act on mechanisms driving the symptoms

US Preventive Services Task Force
Statins Recommendation Statement

Regulation
(Adults aged 40-75 with a history of CVD or DM and CVD risk of 7.5% or higher)
Recomm. Interventions to reduce CVD risk.

Age-wise:
(Adults aged 40-75 who are at moderate risk of CVD and have total cholesterol levels of 150-199 mg/dL and a low HDL of 40-49 mg/dL)
Recomm. Interventions to reduce CVD risk.

Population
Adults who are at low risk of CVD

Recommendation
No recommendation

Are cause/effect relations patient-independent?
Is the PK/PD patient-independent?

Some things we know are not universal

Are cause/effect relations patient-independent?
Is the PK/PD patient-independent?

Some things we are trying to figure out

Personalized medicine

“steering the right patients to the right drug at the right dose at the right time”

The Path to Personalized Medicine
Margaret A. Hamburg, M.D., and Francis S. Collins, M.D., Ph.D.

Personalized medicine

“medical decisions, practices, interventions and/or products being tailored to the individual patient based on their predicted response or risk of disease”

Personalized medicine

The use of genomic information – in addition to family history, lifestyle, and environmental factors – to customize health management. By combining genomic and clinical information, more accurate predictions can be made about a person’s susceptibility of developing disease, the course of disease, and response to treatment.
The origins of personalized medicine

It is more important to know what sort of person has a disease, than to know what sort of disease a person has.

Hippocrates extended Alcmaeon’s doctrine, along with Epedocles’s concept of the four elements, to define four basic humors: sanguis/blood (air), phlegm (water), choler (fire), and melancholia (earth). Choler and melancholia were sometimes referred to as yellow and black bile, respectively. Disease was considered to be the result of an imbalance (dyscrasia) between these humors, and it was the role of the physician and patient to try to reestablish a proper equilibrium (eucrasia). A word we use today, “complexion,” was derived from the practice of complexing the four humors to define the unique characteristics of each individual and their state of health, a practice we might now refer to as phenotyping. We can still classify people today as being “good-humored” or “ill-humored,” as a result of the primary role of the humors in early medical practice.

Early examples of personalized medicine

1957: Reuben Ottenberg reports the first known blood compatibility test for transfusion using blood typing techniques and cross-matching between donors and patients to prevent hemolytic transfusion reactions.

1956: The genetic basis for the selective toxicity of laser beams (“laser”) and the antimetabolite drug trimetrexate is discovered to be a deficiency in the metabolic enzyme, glycero-6-phosphate dehydrogenase (G6PD).

1977: Cytochrome P450 2D6, a polymorphic metabolizing enzyme, is identified as the culprit for causing some patients to experience an “overdose” or exaggeration of the duration and intensity of the effects of desipramine, a drug used for treating hypertension.

The basic tenet of gene-centric personalized medicine

“Using tumor profiling to better understand how patients’ tumors become resistant to cancer therapy. Establishing a repository of laboratory models derived from patient tumor samples that will enable researchers to more thoroughly investigate drug resistance.”

PGx and personalized medicine

https://medicine.iupui.edu/PGx/PGx.html

Leading causes of death in the USA in the early 1900’s

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm4829a1.htm

Leading causes of death in the USA in 2010

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6208a8.htm
Non communicable diseases: Chronic, non-communicable diseases (NCDs) are the number one cause of death and disability in the world. The term refers to a group of conditions that are not mainly caused by an acute infection, resulting in long-term health consequences and often creating a need for long-term treatment and care.

The rise of non-communicable diseases: Mortality


Disability Adjusted Life Years (DALY) is defined by the WHO as "the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability".

NCD and the determinants of health in the 21st century

Psychological distress at any point in the life course is associated with higher cardio-metabolic risk.

Personalized medicine: not just in your genes

- 289 cardiovascular pharmacogenomics studies
- 229 reported positive findings
- 220 unique SNP-drug associations
- 19 confirmed using strict criteria
- 0 recommended for use in clinical practice

"the notion that genetic information is uniquely important in determining the risks and benefits of treatments—is clearly unwarranted and counterproductive to the broadly shared goal of tailoring care to individuals"
Personalized medicine: not just in your genes

“the contribution of genetic discoveries to the clinical management of diabetes and obesity remains limited to the small proportion of cases with monogenic forms of disease”

What is special about non-communicable diseases?

NCD (CVD, Diabetes, Chronic Respiratory Disease, Cancer) are chronic conditions heavily influenced by urbanization (behavioral, environmental, socio-economic factors)

There is no cure for any of these diseases, only symptom mitigation, because it is not clear what the underlying cause it (I think …)

“Indeed, finding a cure for established RA is possibly no more likely than finding a cure for hypertension, where control of organ damage is the only realistic goal of therapy”

The emergent complexities of NCD

Progress

Chronic

Multi-factorial

Co-morbidities
The emergent complexities of NCD

Environmental enrichment (nest making) and oxytocin (brain neuromodulator hormone) comparably promote burn wound healing in isolation reared rats (PLosONE, 4:e5523, 2009)


Expectation of fear HR, inflammation & ANS (PNAS, 112:1248, 2015)

A systems view …

Can personalized medicine help us better describe the state of the patient?
Can personalized medicine transition from disease-centric to patient-centric? In addition to genetics, should it also reflect the evolving dynamics of the patient’s health? If the answer is yes, then how do we achieve this and what complications (technical, ethical, societal, economic) do we foresee?
Patient Centered Approach for Non-Communicable Diseases

Alaina Howe
Department of Chemical Engineering
Rutgers University

16:125:604: Topics in Advanced Biotechnology II
Friday, February 16, 2017

Problems with current approach

- Reactive approach to medicine
- Traditional characteristics of disease condition and ignore the needs of an individual as a complex system.
- Often does not consider socioeconomic, cultural, or psychological factors.
- Demonstrated need for an integrated, patient-centered system to combat disease.

Personalized Medicine

- Allows for treating dynamic, non-linear interactions between different disease components over time.
- Patient-centered approach
  - Identify markers and implement
  - Combinations of treatment that
  - Have been shown to be successful.
- Predictive approach to medicine
  - Primary, secondary, and tertiary prevention
  - P4 - Predictive, personalized, preventative and participatory medicine

Chronic Non-communicable Diseases (NCDs)

- Survival rates for infectious and genetic diseases have improved
- Cardiovascular, cancer, chronic respiratory, and diabetes
  - Disorders of long duration and slow progression
- 36 million annual deaths, set to increase to 53 million in 2030
- 63% of total deaths
- Largely caused by an unhealthy lifestyle, correlated with low socioeconomic status.
  - Caused by complex gene-environment interactions across the lifespan.
- Manifest in different phenotypic appearances, leading to heterogeneity in presentation.

Chronic Obstructive Pulmonary Disease (COPD)

- Characterized by persistent airflow limitation
- COPD will become 5th largest disease burden and greatest cause of death by 2030.
- In the United States, the annual costs for COPD in 2010 were $49.9 billion.
Complexity of COPD

- Numerous pulmonary and extra-pulmonary components
- Currently diagnosed only by the degree of airflow limitation even though significant heterogeneity exists irrespective of this classification:
  - Clinical presentation, physiology, imaging, response to therapy, decline in lung function, etc.
- Identification and subsequent grouping of key attributes of COPD into clinically meaningful phenotypes is needed to guide more effective therapies and management strategies.

Global Strategy for the Diagnosis, Management and Prevention of COPD (GOLD)

- Attempts to reconcile this impersonal approach by stratifying patients based on
  - Severity of airflow limitation
  - Level of symptoms
  - Previous history

Problems with the current GOLD approach

- Current management strategies still only reflect the persistent emphasis on diagnosis.
  - Rules out other serious diseases and symptom-relieving treatments.
- They rely on patient-initiated visits, relief of symptoms, normalization of lung function, assurance that there is no urgent medical crisis, and on prevention and treatment of acute or chronic emergencies as exacerbations.
- Early intervention not a priority
- Only partly fits with patient-centered goals of chronic care.

Proposed Integrative Approach

- In 1999 Leutz proposed a comprehensive approach.
  - Stability and severity of conditions
  - Duration of illness
  - Urgency of intervention
- Stratified patients into groups and proposed a population management model to better meet the needs of patients
  - Leads to a more efficient, patient directed treatment model

Multidisciplinary Management and Treatment of Chronic NCD’s

- Flexible, holistic intervention based on partnering of many fields, not just treatment of symptoms.
  - Physicians, Dieticians, Nurses, Psychologists etc.
- Predict eventual development of NCD, or progression of the disease based on early risk factors
  - Can cater treatment based on what has worked for similar phenotypes

Moving Forward

- Chronic diseases present a large quantitative burden, and the complexity of medicine has increased.
- Humans are dynamic
  - composed of myriad interacting and self adjusting systems, and diseases develop overlapping several systems.
  - Therefore, treatments and management strategies must reflect this.
Acknowledgements

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Student Coordinator: Seul-A Bae
Discussants: Paulina Krzyszczyk, Antoinette Nelson
Fellow presenter: Alison Acevedo

Questions?

Personalized Medicine Discussion

• Do you think it is practical to have truly personalized medicine at the individual level?
  • Challenges?
  • Potential solutions?

• What part of the personalized medicine process do you think will be the most challenging?

• In what ways is personalized medicine already playing out today?

Ethics

• What are the ethical and legal implications to consider?

• How much information regarding your genome is too much information?
  – Would you want to know which diseases you are susceptible to?
  – Would you want your insurance company to know?

• What populations would be disadvantaged?
When do we transition from health to disease state?

Healthcare is mainly reactive, not preventative

Systems Medicine

P4 Medicine: Quantifying Wellness and Hacking Disease

P4 Medicine: Healthcare Reform

Economic opportunities: data processing and storage, health and wellness industry (mobile monitoring)

Democratization of healthcare: internet, mobile devices, connect patients to each other and to online health resources
P4 Medicine: Healthcare Reform Risks

Prediction
- Genetic risk assessments risk ignoring environmental and epigenetic factors

Prevention
- Revert to reproductive control, eugenics, “socially valued enhancements”

Personalization
- Establishing a genetic social hierarchy

Participation
- Poor decision making of the user overwhelmed with excessive information

Payment
- How will the insurance structure change with disease predictive technologies? Premium considerations rely on genetic predisposition?

Improving Technologies in P4 Healthcare

- Family Genome Sequencing as part of Medical Record
  - Osteoporosis resulting from vitamin D transporter deficiency

- Proteomics and Metabolomics Analyses
  - Institute for Systems Biology provide open sourced protein assay database
  - Seek to develop metabolic monitoring requiring small samples of body fluids for molecular analysis

- Single-cell and Induced Pluripotent Stem Cell Analyses
  - Mixtures of cell types used to understand fundamental biological or disease mechanisms
  - iPSC useful for individual mechanisms of disease progression

The 100K Project

- Employ digital devices to continuously measure physiological parameters
- 4x per year clinical assessment of blood metabolite, gut microbiome, WBC epigenetics, salivary cortisol levels
- Develop assays to monitor immune responses and inflammation
- Data mining to understand development of health within individuals for 20 to 30 years

Monitor 100K well patients, observing changing health, disease state transition and treatment application.

References


Biocompatibility

Adv Biotech
March 3, 2017
Jay C. Sy, Ph.D.

Figures primarily from Biomaterials Science: An introduction to Materials in Medicine, 3rd ed.; Ratner, Hoffman, Schoen, and Lemons

Big picture questions
• How do cells engage with synthetic/non-biological materials?
• How do cell-material interactions evolve over time? When/how can we intervene?

How would you define biocompatibility?
• Non-reactive
• Non-toxic
• Non-immunogenic
• Non-carcinogenic
• Non-thrombogenic
• Non-irritant

Bioinert
“Do no harm.”

Traditional view focused on patient safety

DF Williams, On the mechanisms of Biocompatibility, Biomaterials 2008

How would you define biocompatibility?

“Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimising the clinically relevant performance of that therapy.”

How do cells stick to “stuff”? Hint:
Synthetic surfaces need a protein interface to interact

- Cells have two main classes of adhesion proteins
  - Integrins: cell-ECM interactions
  - Cadherins: cell-cell interactions
- Blood/Serum proteins associate rapidly with implant surfaces
  - Common proteins include fibrinogen, fibronectin, vitronectin

Thermodynamics drive protein adsorption

Surface energy affects protein interactions

Protein-surface affinity affected by many variables

Classical Foreign Body Response
Macrophage evolution in foreign body response

"Frustrated Phagocytosis"

Are controlling macrophages the answer?
Size- and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates

Anderson, et al.

Topics in Advanced Biotechnology
Dr. Jay Sy
March 3, 2017
Presenter: Sonia Yevick

Implantable Biomaterials

- What are they used for?
  - Replace damaged body part
  - Improve function
  - Aid diagnosis, healing, treatment

Type I Diabetes

Potential treatment

- Glucose
- Insulin
- Transplanted healthy islet cells
- Recognition and fibrosis

Implantable Biomaterials

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  - Replace damaged body part
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  - Aid diagnosis, healing, treatment

Type I Diabetes

Potential treatment

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- Transplanted healthy islet cells
- Recognition and fibrosis

Problem & potential solutions

- Potential solutions:
  - Surface chemical changes – e.g. limiting protein fouling
  - Surface texture changes – e.g. porosity
  - Changes in material type and geometry

Device failure

Patient discomfort

Study design

Q1: Does sphere size affect FBR?

Conclusion: Less FBR with larger spheres

Study design

Q2: Function of surface area?

Conclusion: Medium spheres clumped and fibrosed, large spheres clear and not (highly) fibrosed
Q3: Is it just a function of kinetics?

Conclusion: Small spheres clumped and fibrosed, large spheres clear and not (highly) fibrosed

Q4: What about other materials?

Conclusion: Larger spheres had less cellular deposition in all cases (less thick layer)

Materials:
- SLG20 alginate
- LF10/60 alginate
- Stainless steel
- Glass
- Polycaprolactone
- Polystyrene

Q5: What about higher order species?

Conclusion: Larger spheres produce less FBR in non-human primates (both subQ and IP)

Q6: Greater biocompatibility = greater functionality of implanted biomaterial?

Conclusion: Mice with larger encapsulation spheres were cured for longer than those with smaller

Q7: A deeper look at the immune response

Conclusion: Fewer macrophages gather by larger spheres
Macrophages

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Type</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 7</th>
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<td>v</td>
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p values: significance between MF type in each compartment/sphere size vs. mock surgery (from nanostring data)

Summary/Critiques

Summary

- Contrary to prior assumptions, larger sphere size leads to less of a foreign body response, less fibrosis, and extended functionality of implanted encapsulated Islet cells
- Experiments mostly done in intraperitoneal space

Critiques

- Contradiction/confusion in the blood glucose control experiment
- Macrophage phenotypes – need kinetic data
- No recovery percentages
- Inconsistent sizes/studies (communication)

Acknowledgements

Thank you!

- Dr. Jay Sy
- Sal Ghodbane
- Anton Omelchenko
- Trevan Locke
- Peter Swiatkowski
- Biotechnology Training Program

Specific Questions

- Are you convinced PCBMA is a good candidate for new medical devices? What else would you want to see?
- Why do large spheres have lower amounts of cellular deposition? Does it have to do with sphere packing?
- Why quantify macrophage activation if large spheres have no cellular deposition to begin with? Should the data then be normalized and represented as percentage?

Broad Questions

- Apparently this was the first paper to investigate the effect of sphere diameter on biocompatibility. Are there any variables in your field that people might be taking for granted?
- Why should we care about this? What broad applications does either of these models have?
- How much is enough when exploring something like a new material? For example, is a short research letter with characterization enough or should we strive for more complete works with characterization and some application?
- What is biocompatibility?

Study | Assays | Size (mm diameter) | Material(s) | Model/locati-

- 1 – sphere size
  - Immunostaining, q-PCR, Western blot
  - Rat
  - C57BL/6 mice - intraperitoneal

- 2 – surface area
  - Phase contrast
  - 0.5, 1.5

- 3 – kinetics
  - Phase contrast
  - 0.3, 1.5

- 4 – materials
  - Immunostaining, flow cytometry, q-PCR
  - Multiplexed inflammatory mouse cytokine profile
  - SLG20 alginate, LF10/60 alginate, stainless steel, glass, polycaproleactone, polyurethane

- 5 – rat
  - Immunostaining, q-PCR
  - 0.5, 2
  - Sprague-Dawley rats - intraperitoneal
Zwitterionic hydrogels implanted in mice resist the foreign-body reaction

Lei Zhang1, Zhiqiang Cao1, Tao Bai1, Louisa Carr1, Jean-Rene Ella-Menye1, Colleen Irvin2, Buddy D Ratner1,2 & Shaoyi Jiang1,2

Neuroscience Graduate Program
March 5th, 2017

Encapsulation of Implanted Materials & Devices
- Materials and devices used for drug delivery, prosthetics and tissue engineering are identified as foreign by body’s immune system
- Results in encapsulation by dense collagenous capsule
- Capsules are impermeable to molecules in local environment
- Implant function is disrupted leading to tissue distortion or pain


Foreign Body Reaction
- Protein adsorption on to the implant may lead to the initiation of foreign body reaction
- Macrophages fail to phagocytose the implant
- Fuse to create foreign body giant cells and release cytokines
- Fibroblasts generate dense avascular layer of collagen on top of the implant encapsulating it

Materials Resulting in Foreign Body Reaction
- Teflon, polyurethane, silicone rubber, polyethylene, poly(methyl methacrylate), poly(2-hydroxyethyl methacrylate) (PHEMA), poly(ethylene glycol) (PEG), Dacron, gold, titanium and alumina

Zwitterionic Materials
- Contain equivalent cationic and anionic groups and maintain overall charge neutrality
- Part of class of ultra-low-fouling materials
- Includes phosphorylcholine, sulfobetaine and carboxybetaine


Hypothesis
- Material which does not undergo nonspecific protein adsorption may prevent implant encapsulation.
- Property termed non-fouling
- Most commonly utilized non-fouling or low-fouling materials are PEG and PHEMA
- PEG and PHEMA are both eventually encapsulated when used in implants

Carboxybetaine

- Poly(carboxybetaine methacrylate) (PCBMA) hydrogels made from carboxybetaine monomer (CBMA) and carboxybetaine cross-linker (CBMAX) are considered ultra-low-fouling
- Shown to adsorb <0.3 ng/cm² proteins in blood serum & plasma
- Lead to less cell adhesion compared to PHEMA

Macrophage activation

- Macrophage activation ranges from classical activation (more pro-inflammatory) to alternative activation (more anti-inflammatory & pro-healing)
- Pro-inflammatory macrophages recruit other macrophages through the release of inflammatory cytokines
- Anti-inflammatory macrophages induce angiogenesis & tissue remodeling
Hypothesis

- The two varying foreign body reactions triggered by the PCBMA and PHEMA hydrogels may be due to contrasting macrophage activation
- Authors examined macrophage expression at hydrogel-tissue interface

Macrophage Activation

- Immunostained for pro-inflammatory markers (labeled green):
  - inducible nitric oxide synthase (iNOS)
  - interleukin-1 receptor 1 (IL-1R1)
  - tumor necrosis factor-α (TNF-α)
  - C-C chemokine receptor type 7 (CCR7)
  - IL-12 p35 subunit
- Anti-inflammatory markers (labeled blue):
  - macrophage mannose receptor (MMR)
  - arginase-1 (Arg-1), IL-10
  - scavenger receptor B I/II (SR-BI/II)
  - found in inflammatory zone (FIZZ1)

Hydrogel Mechanical Properties

- PCBMA & PHEMA hydrogel samples had similar mechanical properties
- Compressive modulus of PCBMA hydrogels ranged from ~0.16 megapascal (MPa) to 60 MPa
- PHEMA hydrogels ranged from ~0.29 MPa to ~70 MPa
Summary

- Zwitterionic hydrogels made from carboxybetaine monomer & cross-linker diminish the foreign body reaction
- PCBMA prevented the generation of collagenous capsule up to 3 months and promoted angiogenesis in nearby tissue
- Zwitterionic hydrogels may play important role in improving the function of drug delivery devices, tissue scaffolds and artificial organs.

Critiques

- All quantitative analysis performed on immunofluorescence data
- Could have used ELISA to strengthen findings
- Lack of specific application in paper, only general implantation of hydrogels

Acknowledgements

- Biotechnology Training Program
- Dr. Jay Sy
- Sal Ghodbane
- Peter Swiatkoski
- Trevan Locke
- Sonia Yevick

Image References

   d00084375-medium.png
   d00134349-medium.png
Humanized Mouse Models for Advanced Pre-clinical Studies

Topics in Advanced Biotechnology

Andrew Zloza, MD, PhD
Section Chief, Surgical Oncology Research, Rutgers Cancer Institute of New Jersey

So what exactly is a humanized mouse? …

Consider that …

"Essentially, all models are wrong …

… but some are useful"

Advantages

Disadvantages

George E. P. Box

https://en.wikipedia.org/wiki/George_E._P._Box

You vs mouse

Humanized mice are particularly useful…. 

- Conditions where sufficient alternative models do not exist
  - Where animal models (e.g., mice, monkeys, etc.) do not have complimentary disease
    - e.g., Human Immunodeficiency Virus (HIV)-induced non-AIDS-defining cancers
  - Where the treatment only affects human cells (e.g., not mouse)
    - e.g., Oncolytic virus therapy utilizing viruses not infecting mice
  - Where testing of human-grade drugs (e.g., GMP-quality drugs created for patient treatment) is desired in a live host
    - e.g., using GMP-grade anti-human PD-1 antibody

Common models of cancer

- Mouse cell line in mouse
  - Syngeneic, injected, immunocompetent
- Genetically driven mouse tumor
  - GE-MM, immunocompetent
- Human cell line in mouse
  - Humanized, injected, immunocompromised
- Patient-derived xenograft
  - Humanized, immunocompromised
- Double-humanized mouse
  - Humanized, immune-reconstituted
**What are PDX models?**

- Subset of humanized mice: engrafted with components from patients
- Used in infectious disease, transplant/GVHD, and cancer

**Base = immunodeficient mouse**

<table>
<thead>
<tr>
<th>Engraftment of patient-derived tissue into a mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base = immunodeficient mouse</td>
</tr>
<tr>
<td>Tumor dissociation to single cells</td>
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<tr>
<td>Tumor injection</td>
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<tr>
<td>Therapy</td>
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</tbody>
</table>

**How are PDX models created?**

- Cells or tissues derived from patients are engrafted

**So what are the advantages and disadvantages of PDX creation?**

**Advantages:**
- Unbiased representation/sampling of the whole tumor
- Large number of mice can be challenged with primary tumor cells (especially within a supportive/collagen matrix)
- Tumor microenvironment is preserved (hypoxia, acidity, cell-cell interactions, tissue architecture)
- May better reconstitute the immune component

**Disadvantages:**
- Dissociation capabilities and forces may equally limit cell types
- Formation of mouse-human hybrid tumor (increased with passages)
- Tumor microenvironment is not preserved

**How are PDX models created?**

- Cells or tissues derived from patients are engrafted
Do PDX transplanted tumors allow study of metastasis?

Breast cancer
Transplant
Mammary fat pad
NOD/SCID

Pancreatic cancer
Transplant
Subcutaneous
NOD/SCID

DeRose et al. Nature Medicine, 2011

Can PDXs be expanded and passaged?

Pancreatic cancer
Transplant
Subcutaneous
NOD/SCID

Breast cancer
Transplant
Mammary fat pad
NOD/SCID


Prognostic value: Metastasis and Survival

Future of PDXs
• Combination of patient tumor tissue + autologous immune reconstitution

Double-humanized mouse model
– Created by:
  • Humanization 1: adoptive transfer of peripheral blood cells from a cancer patient
  • Humanization 2: challenge with tumor from the same (autologous) cancer patient

NCI
Administrative supplements (for CCSGs, SPOREs, NCTN, and UM1 grantees) to support collaborative research efforts to enhance preclinical drug development and preclinical clinical trials utilizing patient derived xenograft (PDX) models. — issued May 2, 2016

Malaney et al., Cancer Letters, 2013

Zhou et al., Cancer Letters, 2014

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Malaney et al., Cancer Letters, 2013

Zhou et al., Cancer Letters, 2014
**Result:** consistent model for evaluation of the tumor and anti-tumor immune responses

**More applications of this (and similar) models**

- Normal tissues benefit from immune reconstitution also!

  - [Image: mouse model showing immune reconstitution](unpublished and confidential)

- Future cardiovascular applications?

  - [Image: diagram showing myocardial infarction](unpublished and confidential)

**Result:** strong immune reconstitution

**Can humanization be improved?**

**Other applications for humanized mouse models**

- Immune reconstitution from patients with (vs without) a particular disease can mimic the disease

**Consider that …**

“Essentially, all models are wrong …

…but some are useful”

- [Image: George E. P. Box](https://en.wikipedia.org/wiki/George_E._P._Box)
Development and function of human innate immune cells in a humanized mouse model


Presented by Jenna Newman
March 31, 2017

Introduction to the MITRG/MISTRG humanized mouse model

| Deficiency in recombination activating gene 2 | impedes normal development of B and T cells |
| Deficiency in interleukin-2 receptor subunit gamma | failure of T, B and NK cells to mature |

+ |

M-CSF  GM-CSF  IL-3  thrombopoietin  SIRPα

MITRG  MISTRG

Engraftment of human CD45+ cells in MITRG and MISTRG mice

Mice were NOT irradiated and administered fetal liver-derived CD34+ cells via intrahepatic injection

MISTRG

MITRG and MISTRG mice exhibit engraftment of myeloid lineages

Myeloid cell subset diversity is recapitulated in humanized MITRG and MISTRG mice

Engrafted monocytes are responsive upon immune challenge in MITRG and MISTRG mice
Natural killer (NK) cells are observed in MISTRG mice

High expression of IL-15 and IL-15Rα by monocytes in MISTRG and MISTRG mice → NK engraftment

Clodronate = drug used to deplete phagocytic cells

Human NK cells exhibit full functionality in MISTRG mice

*MITRG mice have significantly fewer peripheral NK cells than MISTRG mice, presumably due to lack of human SIRPα. MITRG mice were not tested for NK function

Adaptive immunity is compromised in MITRG and MISTRG mice

B cells in MITRG/MISTRG mice are found at lower frequency than observed in humanized NSG mice and exhibit immature phenotype

T cells in MITRG/MISTRG mice are reduced relative to that observed in humanized NSG mice, but functional

MISTRG mice exhibit human-like pattern of tumor growth and immune infiltration

Conclusions and Limitations

- Successful engraftment of human myeloid lineages was observed in MITRG and MISTRG mice → major advance in the development of humanized mouse models
- Major Limitation #1: MITRG and MISTRG mice are prone to anemia → short experimental window
- Major Limitation #2: Engraftment of B and T cells is compromised in this model

Acknowledgements

- Andrew Zloza, M.D. Ph.D.
  - Jeremy Anderson
  - Jose James
  - Kirsten Svane
  - Corina White
Development and function of human innate immune cells in a humanized mouse model
Presented by Jenna Newman
March 31, 2017

Introduction to the MITRG/MISTRG humanized mouse model

- Deficiency in recombination activating gene 2 (Rag2) impedes normal development of B and T cells
- Deficiency in interleukin-2 receptor subunit gamma (Il2rγ) failure of T, B and NK cells to mature

M-CSF
GM-CSF
IL-3
thrombopoietin
SIRPα

Engraftment of human CD45+ cells in MITRG and MISTRG mice

Mice were irradiated and subsequently administered fetal liver-derived CD34+ cells via intrahepatic injection.

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  - Jeremy Anderson
  - Jose James
  - Kirsten Svane
  - Corina White
Why do we want to edit genomes?

- Treatment of inheritable diseases through gene therapy
- Treatment of acquired genetic diseases (cancer mostly, perhaps ageing)
- Faster, easier and safer (and cheaper) GMOs
- Accelerate genetics research
Using HDR as an \textit{in vivo} genome editing tool \textbf{“ends out”}

In yeast, 10-50% of marker + cells are correct, the rest are random insertions. In mammals, correct transformants are much less frequent.

Using HDR as an \textit{in vivo} genome editing tool \textbf{“chromosome capture”}

Target size vs number of sites in human genome

Site-specific endonucleases
- Restriction enzymes?
- Non-specific endonuclease that can be targeted to arbitrary targets

Programmable Site-specific endonucleases
- Zinc-finger nucleases (ZFN)
- TAL-effector nucleases (TALEN)
- CRISPR
**Programmable Site-specific endonucleases**

**ZFN and TALEN**

- Protein DNA binding domain engineered to bind target sequence
- Sequence-inspecific endonuclease

**Programmable Site-specific endonucleases**

**CRISPR**

- CRISPR/Cas9
- sgRNA
- Recombinant Cas9 + synthetic sgRNA
- sgRNA expression vector + Recombinant Cas9
- sgRNA and Cas9 expression vector

**The CRISPR defense mechanism**

- Stage 1: Foreign DNA acquisition
- Stage 2: CRISPR RNA processing
- Stage 3: RNA-guided argonucleic acid interference

**Xenbase.org**
CRISPR/Cas9 genome editing

- HDR
- NHEJ

CRISPR/Cas9 genome editing Limitations

- PAM sequence needed (3bp, not a big deal)
- Cas9 is somewhat toxic
- Possible off-target effects
  - (target base-pairing allows some slack, ~8-10 bp is enough to see some binding)
  - Cleavage efficiency is variable between targets
    - Sequence dependence
    - Chromatin accessibility

CRISPR/Cas9

Nuclease deficient Cas9 (dCas9)

CRISPR/Cas9 Gene Drives

Doubles target sequence size: practically eliminates off-target effects
Ethical implications of widely accessible, inexpensive, flexible genome editing

Too many for today
What do YOU think?

THANK YOU

Questions?
Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage

Larry Cheng
April 28, 2017

Need for increasing HDR efficiency and suppressing NHEJ
- CRISPR caused double strand breaks lead to competing mechanisms
  - HDR: homology directed repair
  - NHEJ: non-homologous end joining
- Indels are more abundant than gene replacement
  - Inefficient point mutation correction (~0.1-5%)

Base editing strategy
- Catalytically dead Cas9
  - D10A
  - H840A
- Cytidine deaminase

BE1 mediates C → U conversion with an activity window of ~5 nucleotides
- BE1 (1st generation base editor)
  - Dead Cas9
  - APOBEC1 (cytidine deaminase)
  - XTEN (linker)

Base editing efficiency affected by protospacer sequence

BE1 is processive
- protospacer and PAM sequence: 5'-TTCCCCCCCCGATTATTATGG-3'
<table>
<thead>
<tr>
<th>protospacer</th>
<th>% of total reads</th>
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<tbody>
<tr>
<td>TTTTTTTTT</td>
<td>62.4</td>
</tr>
<tr>
<td>TTTTTTTTC</td>
<td>18.2</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>13.4</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>3.3</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>0.8</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>0.3</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>0.3</td>
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<tr>
<td>TTTTTTTTT</td>
<td>0.3</td>
</tr>
<tr>
<td>TTTTTTTTT</td>
<td>0.3</td>
</tr>
</tbody>
</table>
BE1 conversion efficiency diminished in human cells

BE2 demonstrates “threefold higher” conversion efficiency in human cells
- BE2 (2nd generation base editor)
  - Fused uracil DNA glycosylase inhibitor to C-terminus of BE1
  - Inhibits base excision repair initiation

BE3 may push MMR to favor conversion
- BE3 (3rd generation base editor)
  - Restores catalytic His (AB40H) in Cas9
  - Nicks the unedited strand to induce eukaryotic mismatch repair (MMR) system to excise base in opposite strand

Greater conversion but greater indel formation in human cells with BE3

Base editing persists over cell divisions
Off target conversions outside the protospacer

APOE4 conversion to APOE3r in mouse astrocytes by BE3

p53 Y163C correction in breast cancer cells by BE3

284 potentially actionable variants by base editors

Summary
- Base editing technology
  - Converts C to T
  - Located certain distance from a PAM sequence
- Limitations
  - Efficiency is not 100%
  - Off-target conversions
  - Not every clinical variant is actionable
  - Introduction of indel mutations
  - In vitro experiments

Acknowledgements
- Dr. Mikel Zaratiegui
- Chris Lowe
- Ilija Melentijevic
- Evelyn Okeke
- Eve Reilly
- Rutgers Biotechnology Training Program (NIH T32 GM008339)
A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae

Hammond et. al., Nature Biotechnology, 07 December 2015

What is a gene drive?

Anticipated transmission of the CRISPR-Cas9 mediated gene drive in A. gambiae

Identification of three putative female fertility genes in A. gambiae

Orthology Sterility Index

AGAP005958
AGAP01377
AGAP007280

Gene editing using CRISPR/Cas9

Generation of 3 ‘docking lines’ by homology-directed repair (HDR)
Gene disruption by HDR causes recessive female sterility

Replacement of the docking lines with a CRISPR homing construct by RMCE

CRISPR homing constructs exhibit gene drive activity

CRISPR homing constructs significantly impair female fertility

The CRISPR<sup>h</sup> allele can spread in a caged population

Gene drives: consequences and pitfalls

- Easily thwarted by evolution
Gene drives: consequences and pitfalls

- Easily thwarted by evolution
- Unknown ecological effects
- Unpredictable

Questions?

Acknowledgements:
Dr. Mikel Zaratiegui
Chris Lowe
Evelyn Okeke
Ilija Melentijevic
Larry Cheng
Rutgers Biotechnology Training Program
NIH T32 GM008339

Discussion Questions

- What are the ethical concerns surrounding gene drives?
- Can gene drives be adapted as a therapeutic approach in humans?
- What diseases would be good candidates for targeted base pair repair?
- What are some other potential applications for engineered Cas-9 proteins?
<table>
<thead>
<tr>
<th>Date</th>
<th>Week</th>
<th>Session Objective</th>
</tr>
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<td>1/25/2017</td>
<td>1</td>
<td><strong>Introduction:</strong> Familiarize the student with course logistics, technology entrepreneurship and the nature of innovation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Course overview</td>
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<td>• Entrepreneurship defined</td>
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<td>• Emerging technologies</td>
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<td>• Profile of an entrepreneur</td>
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<td>• Entrepreneurial types/teams</td>
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<td>• Entrepreneurial risks</td>
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<td>• Staged entrepreneurial process</td>
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<tr>
<td>1/25/2017</td>
<td>2</td>
<td><strong>Analyze the Opportunity:</strong> Innovate and Create the Vision</td>
</tr>
<tr>
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<td>• Observation, problem and need identification</td>
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<td>• Needs filtering</td>
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<td>• Ideation and brainstorming</td>
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<td>• Concept screening</td>
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<td>• Importance of documentation</td>
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<td>• Review of venture project technologies and team assignments</td>
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<tr>
<td>2/1/2017</td>
<td>3</td>
<td><strong>Analyze the Market:</strong> Analyze the Market and Build a Plan, Prepare Industrial Analysis</td>
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<td>• Innovation types and frameworks</td>
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<td>• Initial innovation assessment</td>
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<td>• Market analysis and planning</td>
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<td>• Market segmentation</td>
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<td>2/8/2017</td>
<td>4</td>
<td><strong>Analyze Competitive Position, Market Forces</strong></td>
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<td>• Competitive analysis</td>
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<td>• Porter’s 5 market forces</td>
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<td>2/15/2017</td>
<td>5</td>
<td><strong>Profile the Product/Service</strong></td>
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<td>• Profile essentials: product, pricing, place, promotion</td>
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<td>• Create a brand</td>
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<td>2/22/2017</td>
<td>6</td>
<td><strong>Communicate the Opportunity:</strong> Build “The Pitch”</td>
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<td>• Communication guidelines</td>
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<td>• Pitch elements</td>
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<td>3/1/2017</td>
<td>7</td>
<td><strong>Implement, Scale &amp; Harvest the Venture:</strong> Setting Up the Company, Team Management, Venture Exit</td>
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<td>• Business planning outline</td>
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<td>• Setting up a company</td>
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<td>• Managing the team</td>
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<td>• Exiting the venture</td>
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<td>3/8/2017</td>
<td>8</td>
<td><strong>Protect the Innovation:</strong> Determine best protection method, File necessary documentation</td>
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<td>• IP protection overview</td>
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<td>3/15/2017</td>
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<td>SPRING BREAK</td>
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<td>3/22/2017</td>
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<td><strong>Acquire Financial Resources</strong>: Secure Early Stage and Growth Funding</td>
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<td>- Valuation overview</td>
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<td>- Funding sources: equity and non-equity sources</td>
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<td>- Angel, Venture Capital funding</td>
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<td>3/29/2017</td>
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<td>Tim Maguire: VascuLogic; the commercialization journey</td>
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</table>
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, PhD, Tim Maguire, PhD, and Martin Yarmush MD, PhD

Grading:
- 25% Class Participation
- 30% Homework Assignments
- 35% Research Paper
- 10% Short Presentation

Class Preparation: Reading material for each session can be found on the course website:

- 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 2016:
20-Jan: (A) Introduction to Course Objectives
(B) Science-Driven Business: Examples in the Pharma Industry
Tim Maguire and Kristen Labazzo, Rutgers University

27-Jan: Discovery and Careers in Biotechnology: A Case Study- Kambiz Shekdar, Rockefeller University

03-Feb: Extracting the Full Potential of Single Use for Biologics and Vaccines - David Pollard, Merck

10-Feb: Preparative Chromatography for the Purification of Therapeutic Proteins - Antonio Ubiera, GlaxoSmithKline
17-Feb: Working the Science: Overcoming Issues in Biopharmaceutical Drug Product Development – Charlene Brisbane, George Crotts, GlaxoSmithKline

24-Feb: The Future of Medical Innovation: A History - Bob Goldberg, Center for Medicine in the Public Interest (CMPI)


09-Mar: Mammalian Cell Culture Scale-Up for Monoclonal Antibody Production - Gregory Russotti, Celgene Cellular Therapeutics

16-Mar: SPRING BREAK

23-Mar: Live Virus Vaccine Production - Gregory Russotti, Celgene Cellular Therapeutics

30-Mar: DNA Vaccines Product Development and New Approaches to Cancer Immunotherapy - Niranjan Sardesai, Inovio

06-Apr: The Hurdles and Benefits of In vivo Relevance in Regenerative and Drug Screening Platforms - Carlos Caicedo, Orthobond

13-Apr: “That Can’t be Right!” - Real Data in Cell Therapy Development - Brian Murphy, Celgene Cellular Therapeutics

20-Apr: Development of Cell Based Therapies - Michael Daley, Cognate Consultants

27-Apr: OPEN

Homework:
- Questions will be posed by the speakers related to articles 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 in hard copy form or submitted to the Sakai website

Presentation:
Each student will have the opportunity to more thoroughly understand a particular course topic through the review of contemporary literature and presentation of a particular article of interest. The individual presentation is intended to expose students to a greater quantity of literature and gain a better understanding how areas of focus are evolving. We encourage you to show preference to topics of interest but recognize that presentations will need to be equally distributed across the class.

Term Paper:

Due date: Wednesday, 27-Apr (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.
<table>
<thead>
<tr>
<th>Week</th>
<th>Topic</th>
<th>Reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Overview and Descriptive Statistics.</strong> Type of data, graphic presentation, central tendency and dispersion, introduction to R, introduction to GraphPad Prism.</td>
<td>1 Ch. 1,2</td>
</tr>
<tr>
<td>2</td>
<td><strong>The Peanut Lab I.</strong> 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.</td>
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<tr>
<td>3</td>
<td><strong>Probability and Distributions.</strong> Probability, conditional probability, binomial and normal distribution.</td>
<td>1 Ch. 3,4</td>
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<td>4</td>
<td><strong>Estimation.</strong> Sampling distribution, confidence interval (population means and proportions), sample size estimation based on proportions.</td>
<td>1 Ch. 5,6</td>
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<tr>
<td>5</td>
<td><strong>Hypothesis Testing.</strong> 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.</td>
<td>1 Ch. 7</td>
</tr>
<tr>
<td>6</td>
<td><strong>The Shell Lab.</strong> 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.</td>
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<td>7</td>
<td><strong>Analysis of Variance.</strong> Comparisons between and among means, multiple comparisons.</td>
<td>1 Ch. 8</td>
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<td>8</td>
<td><strong>Correlation and Regression.</strong> Correlation and simple linear regression.</td>
<td>1 Ch. 9</td>
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<td>9</td>
<td><strong>Multiple Linear Regression and Logistic Regression.</strong> Multiple linear regression, model building and diagnosis, logistic regression.</td>
<td>1 Ch. 10,11</td>
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<tr>
<td>10</td>
<td><strong>Analyze my Data Lab.</strong> Students will analyze data that they have generated using R and GraphPad Prism to analyze and graphically display the results.</td>
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<tr>
<td>11</td>
<td><strong>Nonparametric Statistics.</strong> Sign test, Wilcoxon sign rank test, Wilcoxon rank sum test, Kruskal-Wallis test.</td>
<td>1 Ch. 13</td>
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<td>12</td>
<td><strong>Survival Analysis.</strong> Kaplan-Meier procedure, Log-rank test, Cox proportional hazard model.</td>
<td>1 Ch. 12</td>
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<td>13</td>
<td><strong>Biostatistics in the Genomic Age.</strong> Microarray data analysis.</td>
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<tr>
<td>14</td>
<td><strong>Reading the Scientific Literature.</strong> Use of statistical analysis in the scientific literature, misuse of statistical analysis in the scientific literature.</td>
<td>Handouts</td>
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<tr>
<td>15</td>
<td><strong>Student Presentations.</strong> Students will present preliminary statistical design and data analysis plan for their thesis projects.</td>
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School of Engineering
Special Problems IV: Professional Preparedness in Biotechnology
16:125:624:B1 (3 credit)

Course Background/Overview: Although current courses in the typical graduate curriculum appropriately deliver strategic discipline-based learning for life science and engineering students, the broader biotech and health science industry 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. This course 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.

Pre-requisite: Graduate standing in life or biomedical sciences department or related field. UG Juniors, contact Larry Stromberg for Special Permission # at les42@soe.rutgers.edu

Class Dates and Hours: This course will be offered during the first 2017 Summer Session (May 30, 2017 through July 7, 2017), over a 6-week period, on Tuesdays and Thursdays for sessions of 4 hours each (1:00-5:00 PM). 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.

Instructors: Dr. Martin Yarmush, Susan Engelhardt, Subject Matter Expert Guest Lecturers

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
- **Organizations and Partnerships**: internal and external partnerships, negotiating corporate silos
- **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
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.

**Syllabus**

<table>
<thead>
<tr>
<th>Week</th>
<th>Applications in Medical Devices Development (Lecture order subject to change)</th>
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<tbody>
<tr>
<td>Week 1</td>
<td>A) Introduction to Course Objectives</td>
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<td>B) Job Prospects in Medical Devices</td>
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<td>Week 2</td>
<td>Cardiovascular Devices</td>
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<tr>
<td>Week 3</td>
<td>Orthopedic Reconstruction Devices and Bone Void Fillers</td>
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<td>Week 4</td>
<td>Diagnostic Devices</td>
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<td>Week 5</td>
<td>Rehabilitative Equipment</td>
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<td>Week 6</td>
<td>Dental Applications for Medical Devices</td>
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<td>Week 7</td>
<td>Drug/Device Combination Products</td>
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<td>Week 8</td>
<td>Neurovascular Catheter Devices</td>
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<td>Week 9</td>
<td>General Surgical Products and Instruments</td>
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<td>Week 10</td>
<td>Urinary Medical Devices</td>
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<td>Week 11</td>
<td>Wound Closure Products</td>
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<td>Week 12</td>
<td>Polymeric Disposables</td>
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<td>Week 13</td>
<td>Imaging and Remote Surgical Machines</td>
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<tr>
<td>Week 14</td>
<td>Class Presentations</td>
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</table>

The structure of each individual lecture will cover the following format:

- Introduction to the device
- Market for the device
- Design considerations
- FDA Pathway
- Clinical Trial Requirements
- Manufacturing/QA/QC
- Post-Market Considerations (like reimbursement)

In addition to the weekly homework, a final project will be required as follows:

**Medical Device Proposal:**
Each student will 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 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:** how will you get from concept to product?
- **Investment Opportunity:** how much money are you looking to generate? Can the product be reimbursed through health insurance to make it more attractive 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:** what is the endpoint of your product? Sale to a larger company? License select components? Go to market and sell?
- **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:**
Each student 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.
AGENDA

Continental Breakfast........................................... 9:00 am

Introduction....................................................... 9:30 am

Dr. Martin Yarmush

Keynote Address............................................... 10:00 pm

Dr. Saira Jan, PharmD, MS

Poster Session.................................................. 11:00 am

Lunch............................................................... 12:00 pm

Poster Awards and Closing Remarks........ 1:00 pm
Dr. Jan (Saira), is the clinical professor at Ernest Mario school of pharmacy and leads the Horizon Blue Cross Blue Shield of New Jersey pharmacy clinical management program as a Clinical Director with over 17 years of experience in health care management and research and academics. Dr. Jan is responsible for managing Horizon’s pharmacy clinical initiatives including formulary management, Pharmacy and therapeutic Committee (Co-chair) drug reviews, utilization management, drug policy development, specialty pharmacy, and physician education she works closely with the company’s business units, clinical quality and medical management areas to deliver integrated services.

Dr. Jan leads Horizon BCBSNJ delivery of Medicare Part D Medication Therapy management program for MA-PD and PDP customers. She serves as a consultant to PAAD advisory board, AHIP, Academy of Managed Care Pharmacy and Blues association MAP committee and has a national appointment to MEDCAC. Dr. Jan leads the research program for Horizon BCBSNJ. She is involved in collaborative as well as independent outcome research projects with Harvard, Rutgers and other national academic institutes and pharmaceutical industries. She provides consultations to International research and consultancy groups as well different officials in United Kingdom. She has represented Blues association at the Institute of Medicine for patient safety and adverse drug reaction reporting initiatives.

She has successfully led the New Jersey state leading project on childhood Obesity “Shape it Up” for more than 400 elementary school.

Dr. Jan is also the director of residency program in Managed care and has successfully run the program for last 16 years. She also provides leadership for the Rutgers AMCP student chapter and is the faculty advisor for AMCP student chapter.

STUDENT POSTERS
Alison Acevedo  
Biomedical Engineering  
Dr. Ioannis Androulakis

Paulina Krzyszczuk  
Biomedical Engineering  
Dr. François Berthiaume  
Dr. Martin Yarmush

Misaal Patel  
Biomedical Engineering  
Dr. Li Cai

Jeremy Anderson  
Biomedical Engineering  
Dr. Li Cai

Trevan Locke  
Chemical and Biochemical Engineering  
Dr. Stavroula Sofou

Xiomara I. Perez  
Biomedical Engineering  
Dr. Martin Yarmush

Seul-A Bae  
Chemical and Biochemical Engineering  
Dr. Ioannis Androulakis

Christopher J. Lowe  
Biomedical Engineering  
Dr. David Shreiber

William Pfaff  
Biomedical Engineering  
Dr. Charles J. Gatt  
Dr. Michael G. Dunn

Daniel P. Browe  
Biomedical Engineering  
Dr. Joseph W. Freeman

Ileana Marrero-Berríos  
Biomedical Engineering  
Dr. Martin Yarmush

Christopher Rathnam  
Chemistry  
Dr. Ki-Bum Lee

Larry Cheng  
Cellular and Molecular Pharmacology  
Dr. Justin M. Drake

Ilija Melentijevic  
Molecular Biology and Biochemistry  
Dr. Monica Driscoll

Eve Reilly  
Molecular Biology and Biochemistry  
Dr. Mikel Zaratiegui

Mollie Davis  
Biomedical Engineering  
Dr. Martin Yarmush

Sarah M. Misenko  
Molecular Biology and Biochemistry  
Dr. Samuel F. Bunting

Joseph J. Sherba  
Biomedical Engineering  
Dr. Jeffrey D Zahn

Zachary Fritz  
Biomedical Engineering  
Dr. Martin Yarmush

Antoinette G. Nelson  
Biomedical Engineering  
Patrick J. Sinko

Kirsten Svane  
Cell Biology and Neuroscience  
Dr. Bonnie L. Firestein

Salim A. Ghodbane  
Biomedical Engineering  
Dr. Charles J. Gatt  
Dr. Michael G. Dunn

Jenna Newman  
Biochemistry and Molecular Biology  
Dr. Andrew Zloza

Przemyslaw Swiatkowski  
Cell Biology & Neuroscience  
Dr. Bonnie L. Firestein

Madison Godesky  
Biomedical Engineering  
Dr. David Shreiber

Evelyn Okeke  
Biochemistry and Molecular Biology  
Dr. Kiran Madura

Victor M. Tan  
Pharmacy  
Dr. Justin M. Drake

Ryan Guasp  
Cell and Developmental Biology  
Dr. Monica Driscoll

Anton Omelchenko  
Cell Biology & Neuroscience  
Dr. Bonnie L. Firestein

Corina E. White  
Biomedical Engineering  
Dr. Ronke M. Olabisi

Clara Hartmanshenn  
Biomedical Engineering  
Dr. Ioannis Androulakis

Dharm S. Patel  
Molecular Biology and Biochemistry  
Dr. Samuel F. Bunting

Sonia Yevick  
Biomedical Engineering  
Dr. Jay C. Sy
<table>
<thead>
<tr>
<th>Name/Department</th>
<th>Current or Last Known Position</th>
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<tbody>
<tr>
<td>Patricia Darcy, Biochemical Engineering</td>
<td>Associate Professor, Chemical Engineering, Lafayette College</td>
</tr>
<tr>
<td>Frank Goveia, Microbiology</td>
<td>Director Client Services, IntrinsiQ, LLC</td>
</tr>
<tr>
<td>Michael Sacco, Pharmaceutical Sciences</td>
<td>Executive Director, Product Safety, Global Information and Analysis, Novo Nordisk</td>
</tr>
<tr>
<td>Jean Boyer, Biochemical Engineering</td>
<td>Senior Director Analytical Sciences, Inovio Pharmaceuticals Inc.</td>
</tr>
<tr>
<td>Vaughn Cleghon, Microbiology</td>
<td>Associate Professor, Department of Pediatrics, University of Cincinnati</td>
</tr>
<tr>
<td>Ramona Lloyd, Microbiology</td>
<td>President and Principal Consultant, CymReg Consulting, LLC</td>
</tr>
<tr>
<td>Maria Lee, Pharmaceutical Sciences</td>
<td>Research Scientist, Advanced Care Products, Ortho Pharma</td>
</tr>
<tr>
<td>Diane Zimmerman, Computer Science</td>
<td>Technical Writer and Editor, Self-employed, CO</td>
</tr>
<tr>
<td>Carlos Aparicio, Biochemical Engineering</td>
<td>CEO and President, ImmunoSite Technologies, FL</td>
</tr>
<tr>
<td>Nathan Busch, Biochemical Engineering</td>
<td>Attorney-at-Law, Anovus LLC, MN</td>
</tr>
<tr>
<td>Amlan Dutta, Biochemical Engineering</td>
<td>Executive Director, Merck &amp; Co, PA</td>
</tr>
<tr>
<td>Susan Harlocker, Molecular Biology &amp; Biophysics</td>
<td>Patent Agent, McDermott Will &amp; Emery, LLP</td>
</tr>
<tr>
<td>Deena Oren, Chemistry</td>
<td>Manager, Structural Biology Resource Center, The Rockefeller University</td>
</tr>
<tr>
<td>Maura Collins Pavao, Microbiology</td>
<td>Professor, Biology, Worcester State University</td>
</tr>
<tr>
<td>Mark Riley, Biochemical Engineering</td>
<td>Professor &amp; Department Head, Biological Systems Engineering, University of Nebraska</td>
</tr>
<tr>
<td>Connie Schall, Biochemical Engineering</td>
<td>Professor &amp; Graduate Director, Chemical &amp; Environmental Engineering, University of Toledo</td>
</tr>
<tr>
<td>Nancy Sladicka (Iler), Molecular Genetics</td>
<td>Vice President, Client Services Scientific Pathways, Nucleus Global, NY</td>
</tr>
<tr>
<td>Srikanth Sundaram, Biochemical Engineering</td>
<td>President and Chief Scientific Officer, MAIA Pharmaceuticals, NJ</td>
</tr>
<tr>
<td>William Thorpe, Biochemical Engineering</td>
<td>Area Director, Club Z! In-Home Tutoring Services, Winchester, MA</td>
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<tr>
<td>Ashish Upadhyay, Biochemical Engineering</td>
<td>Senior Research Biochemical Engineer, Merck &amp; Co, PA</td>
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<tr>
<td>Kenneth Valenzano, Pharmacology</td>
<td>Senior Vice President, Amicus Therapeutics, NJ</td>
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<tr>
<td>Madhaven Vasudevan, Biochemical Engineering</td>
<td>Vice President, Analytics Solutions, GENPACT, CA</td>
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<tr>
<td>David Odde, Biochemical Engineering</td>
<td>Professor, Biomedical Engineering, University of Minnesota</td>
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<tr>
<td>Paul Olson, Molecular Genetics</td>
<td>President and Co-founder, Kypha Pharma, Inc.</td>
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<tr>
<td>David Powers, Biochemistry</td>
<td>Senior Principal Research Scientist, Abbott BioTherapeutics, CA</td>
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<tr>
<td>Maria Ortiz Rivera, Microbiology</td>
<td>Scientific Support Call Center Leader, GE Healthcare, MA</td>
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<tr>
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<tr>
<td>Amit Roy</td>
<td>Biochemical Engineering</td>
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<td>Myrna Uytingco</td>
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<td>Clelia Biamonti</td>
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<td>David Lamberto</td>
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<td>Greg Russotti</td>
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<td>Hany Michail</td>
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<td>Lori Herz</td>
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<td>Todd Muccilli</td>
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<td>Jane Tjia (Atkins)</td>
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<td>Albert Alexander</td>
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<td>Aquanette Burt</td>
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<td>Elizabeth Shen</td>
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<td>Deanna Thompson</td>
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<td>C. Alves</td>
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<tr>
<td>Scott Banta</td>
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<td>Michele Burley</td>
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<td>Carlos Caicedo</td>
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<tr>
<td>Tim Maguire</td>
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