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Rutgers, The State University of New Jersey  
University of Medical and Dentistry of New Jersey  
BIOTECHNOLOGY TRAINING PROGRAM

The PhD Training Program in Biotechnology at Rutgers, The State University of New Jersey was established in 1989. It is one of the select group of such programs throughout the country funded by the National Institute of Health (NIH). The 2013-14 year marks the 24th 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 2014-15 year, the NIH is providing 8 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.
Courses – Appendix D

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.

**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.

**NIH Website for Under Represented Minorities – Appendix F**

This website provides selected examples of NIGMS-funded National Research Service Award (NRSA) training programs that have had noteworthy achievement in the recruitment and retention of individuals from underrepresented groups. All NIH-funded NRSA training programs are required to demonstrate successful recruitment and retention plans to enhance diversity. The programs listed, and their most effective recruitment and retention strategies, are offered as examples of a larger group of programs that are striving to meet this goal. These examples are intended to inform and encourage the efforts of other training programs.

**Symposium and Orientation – Appendix G**

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 H

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>Name, Degree</th>
<th>Rank</th>
<th>Primary Department Appointment</th>
<th>Role in Program</th>
<th>Research Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androulakis, Ioannis, PhD</td>
<td>Associate Professor</td>
<td>Biomedical Engineering</td>
<td>Mentor</td>
<td>Systems biology, transcription, inflammation</td>
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<tr>
<td>Arnold, Edward, PhD</td>
<td>Professor</td>
<td>Chemistry and Chemical Biology</td>
<td>Mentor</td>
<td>HIV, AIDS, drugs, vaccines, structural biology</td>
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<tr>
<td>Berman, Helen, PhD</td>
<td>Board of Governors Prof</td>
<td>Chemistry and Chemical Biology</td>
<td>Mentor</td>
<td>Structural biology, structural bioinformatics</td>
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<tr>
<td>Berthiaume, Francois, PhD</td>
<td>Associate Professor</td>
<td>Biomedical Engineering</td>
<td>Executive Committee</td>
<td>Regenerative med, metabolic eng, stem cells for skin wounds</td>
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<tr>
<td>Bertino, Joseph, MD, PhD</td>
<td>Professor</td>
<td>Pharmacology</td>
<td>Mentor</td>
<td>Tumor suppressor genes and drug resistance</td>
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<tr>
<td>Bunting, Sam, PhD</td>
<td>Assistant Professor</td>
<td>Molecular Biology and Biochemistry</td>
<td>Mentor</td>
<td>Cell survival and DNA repair in mammals</td>
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<td>Burley, Stephen, MD, PhD</td>
<td>Distinguished Professor</td>
<td>Chemistry and Chemical Biology</td>
<td>Mentor</td>
<td>Structural biology and proteomics</td>
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<tr>
<td>Cai, Li, PhD</td>
<td>Associate Professor</td>
<td>Biomedical Engineering</td>
<td>Mentor</td>
<td>Tissue engineering, stem cells, retinal cells</td>
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<tr>
<td>Copeland, Paul, PhD</td>
<td>Associate Professor</td>
<td>Biochemistry &amp; Molecular Biology</td>
<td>Mentor</td>
<td>Regulation of gene expression at the translational level</td>
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<tr>
<td>Ebright, Richard, PhD</td>
<td>Board of Governors Prof</td>
<td>Chemistry and Chemical Biology</td>
<td>Mentor</td>
<td>Transcription; Antibacterial Drug Discovery</td>
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<tr>
<td>Firestein, Bonnie, PhD</td>
<td>Professor</td>
<td>Cell Biology and Neuroscience</td>
<td>Mentor</td>
<td>Dendrite branching in forebrain and spinal cord neurons</td>
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<td>Freeman, Joseph, PhD</td>
<td>Associate Professor</td>
<td>Biomedical Engineering</td>
<td>Mentor</td>
<td>Repair of musculoskeletal tissues; tissue engineering</td>
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<tr>
<td>Grumet, Martin, PhD</td>
<td>Professor</td>
<td>Cell Biology and Neuroscience</td>
<td>Executive Committee</td>
<td>Control of Inflammation after spinal cord injury</td>
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<tr>
<td>Ierapetritou, Marianthi, PhD</td>
<td>Professor</td>
<td>Chemical and Biochemical Engineering</td>
<td>Mentor</td>
<td>Systems engineering, metabolic engineering</td>
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<tr>
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<td>Biochemistry &amp; Molecular Biology</td>
<td>Mentor</td>
<td>Signal transduction, cancer, insulin, metabolism, T cells</td>
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<tr>
<td>Name/Degree(s)</td>
<td>Rank</td>
<td>Primary Department Appointment</td>
<td>Role in Program</td>
<td>Research Interest</td>
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<td>Kohn, Joachim, PhD</td>
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<td>Chemistry and Chemical Biology</td>
<td>Mentor</td>
<td>Novel biomaterials design, drug delivery, polymer implants</td>
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<tr>
<td>Kramer, Sunita, PhD</td>
<td>Associate Professor</td>
<td>Pathology &amp; Laboratory Medicine</td>
<td>Mentor</td>
<td>Cell migration, signaling, heart and blood vessel development</td>
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<tr>
<td>Lee, Ki Bum, PhD</td>
<td>Associate Professor</td>
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<td>Mentor</td>
<td>Nanomedicine and Controlling stem cell/cancer fate</td>
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<tr>
<td>Lobel, Peter, PhD</td>
<td>Professor</td>
<td>Pharmacology</td>
<td>Executive Committee</td>
<td>Hereditary neurodegenerative diseases, functional genomics</td>
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<tr>
<td>Marcotrigiano, Joseph, PhD</td>
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<td>Structure and function of hepatitis C viral proteins</td>
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<td>Messing, Joachim, PhD</td>
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<td>Molecular biology of plant development</td>
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<td>Millonig, James, PhD</td>
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<td>Moghe, Prabhas, PhD</td>
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<td>Montelione, Gaetano, PhD</td>
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<td>Mentor</td>
<td>Bioinformatics / hybrid structure determination methods</td>
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<td>Mentor</td>
<td>Protein evolution and folding, de novo design of proteins</td>
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<tr>
<td>Olabisi, Ronke, PhD</td>
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<td>Mentor</td>
<td>Tissue eng, regenerative medicine for injury and disease</td>
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<td>Pedersen, Henrik, PhD</td>
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<td>Executive Committee</td>
<td>Plant cell culture, chemical and biochemical fiber optic sensors</td>
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<td>Mentor</td>
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<td>Mentor</td>
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<tr>
<td>Name/Degree(s)</td>
<td>Rank</td>
<td>Primary Department Appointment</td>
<td>Role in Program</td>
<td>Research Interest</td>
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<td>Sofou, Stavroula, PhD</td>
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<td>Co-Director</td>
<td>Structure/function analysis of signal transduction proteins</td>
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<td>Uhrich, Kathryn, PhD</td>
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<td>Mentor</td>
<td>Microfab, polymer synthesis, polymer-cell interfaces</td>
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<td>Welsh, William, PhD</td>
<td>Professor</td>
<td>Pharmacology</td>
<td>Mentor</td>
<td>Drug discovery, computer-aided modeling and design</td>
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<td>White, Eileen, PhD</td>
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<td>Mentor</td>
<td>Oncogenes, tumor suppressor genes, apoptosis, autophagy</td>
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<tr>
<td>Yarmush, Martin, MD, PhD</td>
<td>Monroe Chair Professor</td>
<td>Biomedical Engineering</td>
<td>Director</td>
<td>Tissue engineering, stem cells, applied immunology</td>
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<td>Zahn, Jeffrey, PhD</td>
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<td>Mentor</td>
<td>BioMEMS, microfluidics, medical devices</td>
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<td>Zaratiegui, Mikel, PhD</td>
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<td>Molecular Biology &amp; Biochemistry</td>
<td>Mentor</td>
<td>Chromatin dynamics, transposons, silencing</td>
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## APPENDIX B: BIOTECHNOLOGY TRAINING PROGRAM FELLOWS

<table>
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<tr>
<th>FELLOWS</th>
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<th>ADVISOR</th>
<th>THESIS TITLE/CURRENT RESEARCH</th>
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<td>Chen, Alvin</td>
<td>Rutgers</td>
<td>BME</td>
<td>Yarmush</td>
<td>Image-Guided Robotic System for Autonomous Peripheral Intravenous Access</td>
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<td>Drzewiecki, Kathryn</td>
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<td>BME</td>
<td>Shreiber</td>
<td>Characterization, Fabrication, and Applications of 3-D Thermo-reversible Collagen Hydrogels.</td>
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<td>Faulknor, Renea*</td>
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<td>Berthiaume</td>
<td>Paracrine Factors secreted by Mesenchymal Stem Cells Improve Skin Wound Healing.</td>
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<td>Fitzgerald, Kate</td>
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<td>BME</td>
<td>Firestein</td>
<td>The Role of Brain-Derived Neurotrophic Factor in Modulating Neuronal Network Dynamics</td>
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<td>Ghodbane, Mehdi*</td>
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<td>BME</td>
<td>Yarmush/Zahn</td>
<td>Microdevice for Analysis of Spinal Cord Fluid</td>
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<td>Gray, Andrea*</td>
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<td>BME</td>
<td>Yarmush/Grumet</td>
<td>Activation of Mesenchymal Stromal Cells for the Treatment of Inflammatory Conditions</td>
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<td>CBN</td>
<td>Firestein</td>
<td>The role of NOS1AP in the dendritic growth of human iPSC-derived neurons</td>
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<td>Zaratiegui</td>
<td>Replication fork barriers guide integration and silencing of LTR retrotransposons in <em>Schizosaccharomyces pombe</em></td>
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<td>Rutgers</td>
<td>BME</td>
<td>Nanda</td>
<td>Structural &amp; biophysical characterization of collagen-like peptide based higher order assemblies to generate biomaterials</td>
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<td>Rutgers</td>
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<td>Olabisi</td>
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<td>Pharmacology</td>
<td>Lobel</td>
<td>Engineering TPP1 variants with longer half-lives for enzyme replacement therapy in LINCL</td>
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<td>Rutgers</td>
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<td>Yarmush</td>
<td>Novel In Vitro and In Silico Assay System to Evaluate Skin Sensitization Potential of Chemicals</td>
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<td>Rutgers</td>
<td>BME</td>
<td>Moghe</td>
<td>Multifunctional Nanopolymers to Prevent Artherogenesis</td>
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<td>Locke, Trevan</td>
<td>Rutgers</td>
<td>CBE</td>
<td>Sofou</td>
<td>Fusogenic Anti-PSMA Liposomes For Antivascular Chemotherapy</td>
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<td>RBHS</td>
<td>Cell &amp; Dev Bio</td>
<td>Kramer</td>
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<td>Yarmush</td>
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<td>RBHS</td>
<td>Pharmacology</td>
<td>Welsh</td>
<td>Drug Discovery and Design Using Computational Molecular Modeling</td>
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<td>Melentijevic, Ilija</td>
<td>RBHS</td>
<td>MBS</td>
<td>Driscoll</td>
<td>Investigating the mechanisms underlying neuronal aging in a <em>C. elegans</em> model.</td>
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<td>Yeboah, Agnes</td>
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<td>The use of nanoparticles made of growth factor-ELP fusion proteins to target various aspects of wound healing</td>
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*Underrepresented Minorities

- **CBE** Chemical and Biochemical Engineering
- **BME** Biomedical Engineering
- **CBN** Cell Biology and Neuroscience
- **NCB** Neuroscience and Cell Biology
APPENDIX C: CURRENT RESEARCH, PAPERS PUBLISHED, PRESENTATIONS, AND PATENTS
2013-2014

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<td>Alvin Chen</td>
<td>The first step in many clinical interventions is to establish access to the venous bloodstream; carried out 1.4 billion times each year, venipuncture is the most commonly performed invasive routine in the United States. Unfortunately, the reported rates of needle stick accuracy vary widely across hospitals, especially in challenging settings where the likelihood of success depends heavily on the patient’s physiology and the practitioner’s experience. As a consequence, difficult venipuncture is the leading cause of injury to both patients (1.2 to 2 million injuries per year) and practitioners (400,000 to 1 million injuries per year) in the U.S., and in total is estimated to cost the U.S. healthcare system $4.7 billion per year. These challenges have, in recent years, driven the development of image-guidance technologies to improve the accuracy of cannula placement. Commercial devices use ultrasound or near-infrared detection to enhance vessel contrast; however, these devices leave the cannula insertion – the ultimate determinant of success – to human hands. Image-guided robotic systems have demonstrated clinical efficacy for many invasive surgical routines, but have yet to be translated for the purpose of venipuncture. The <strong>objective</strong> of my dissertation research is to develop a portable, image-guided device that can autonomously perform venipuncture with &gt;95% accuracy in &lt;1 min for patients with difficult venous access. The <strong>central hypothesis</strong> is that the integration of a near-infrared imaging system with a robotically-driven needle will improve vein visibility, cannulation precision, and completion time across a broad range of demographics. To date, a working prototype has been developed and validated in vitro and in vivo. The remainder of my research will focus on improving the accuracy and safety of the device in order to drive its development toward the clinical evaluation. The <strong>rationale</strong> for this work is that the device, once translated, can 1) significantly raise the standard of care for difficult patients, 2) provide substantive cost-savings for hospitals and diagnostic labs, and 3) demonstrate impact in a number of arenas, including pediatric, geriatric, emergency, and military use. The device may also serve as a platform to merge automated phlebotomy with rapid point-of-care diagnostics. Finally, the technology represents a pivotal step in the miniaturization and automation of surgical robotic systems for routine clinical interventions.</td>
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<td>Kathryn Drzewiecki</td>
<td>The current gold standard for patients in need of organs or reconstructive surgery relies on organ transplants and/or cadaveric tissues. Even with these options, many patients reject replacement organs or tissue, which has led to the development of new strategies to design biomaterials that have low immunogenicity. Many have taken to this goal by utilizing rapid prototyping methods with synthetic materials to build 3-D architectures of organs. Though this method is promising, only simple 3-D structures can be fabricated for less complicated surgeries. A material that could naturally integrate into the surrounding tissue that simultaneously supports cell growth would be ideal for a tissue engineered equivalent. Our lab has modified bioactive components of collagen hydrogels by covalently linking peptide fragments to the free amines of the lysines of type-I collagen to evoke specific cell behavior. Specifically, we have used peptide-grafted collagen as a constituent of nerve guidance conduits to support nerve regeneration following spinal and peripheral nerve injuries <em>in vivo</em>. Recently, we synthesized collagen methacrylamide (CMA), which allows for spatiotemporal regulation of bioactive and mechanical properties based on application of UV light. While we utilized this material to create distinct cell microniches to control stem cell behavior, we also developed collagen-based “sponges” for skin replacements and discovered that this material could reversibly self-assemble in a temperature-dependent manner. With this new property, we can fabricate specific 3-D architectures by crosslinking the areas of a hydrogel to ‘keep’ and disassemble the remaining pieces by cooling the gel. The <strong>objective</strong> of this study is to develop a rapid prototyping method for methacrylated collagen for complex engineered tissue equivalents (TEs). The <strong>central hypothesis</strong> is that methacrylated collagen hydrogels can substitute for soft tissues, such as for skin and facial transplants, by 1) recapitulating the 3-D architecture of the tissue, 2) having low...</td>
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immunogenicity, and 3) supporting cell infiltration and growth. Thus far, we have examined the extent to which CMA reversibly disassembles in hopes of targeting the exact mechanism. We have shown that as CMA and type-I collagen self-assemble, fibril size increases, and triple helix structure disappears, potentially due to π-π stacking of tyrosine residues in the telopeptide region. As CMA dis-assembles, fibril size decreases, and triple helix structure returns. We hypothesize that a high lysine content in the C-terminal telopeptide region of type-I collagen is responsible for the reversible self-assembly of CMA. This region typically stabilizes fibril formation, as it forms crosslinks between triple helices. Potentially, with methacrylate groups present, these crosslinks cannot form, and thus at cool temperatures fibrils may dissociate. To begin to utilize CMA as an engineered TE, our specific aims in this study are as follows: 1) to determine the mechanism of CMA thermo-reversible self-assembly; 2) to optimize rapid prototyping methodology to construct CMA TEs; and 3) to analyze cell viability and function in vitro in CMA TEs.

**Renea Faulknor**

Chronic wounds are “stuck” in an inflammatory state characterized by low oxygen and high levels of inflammatory mediators such as inflammatory proteins, white blood cells and bacteria. The hostile environment of chronic wounds readily breaks down growth factors needed to promote wound healing. Recent studies have shown that mesenchymal stem/stromal cells (MSCs), which are non-hematopoietic stem cells, possess “anti-inflammatory” properties. These cells secrete factors that mitigate inflammation and promote wound healing of normal wounds. However, a major limitation with using MSCs is their ability to migrate away from the wound site. Therefore, we have generated a novel bioactive bandage made with alginate to immobilize the MSCs at the wound site. Alginate is an FDA approved material for wound dressings and alginate dressings have shown some therapeutic benefits in chronic wound healing. The **goal** of this project, unlike growth factor depots, is to have the MSCs provide sustained release of soluble factors to the wound and potentially modulate their secretion pattern depending on the wound environment. We **hypothesize** that: (1) encapsulated MSCs are capable of paracrine signaling with macrophages and fibroblasts in the wounds through soluble factors; and (2) this paracrine signaling can enhance wound healing even in the face of impaired blood flow to the wound area and prolonged inflammation in the wound. We plan to test our hypothesis with the following specific aims: (1) to characterize the effect of a simulated chronic wound environment on the viability and secretion pattern of encapsulated MSCs; (2) to characterize the effect of MSC-derived factors on macrophage transition to an anti-inflammatory phenotype and fibroblasts differentiation to myofibroblasts in a simulated chronic wound environment; and (3) to characterize the dynamic interaction between encapsulated MSCs and a chronic wound model. Aims 1 and 2 are completed and I am currently working on a manuscript showing that MSCs can reverse hypoxia suppression of α-smooth muscle actin expression in human dermal fibroblasts. Alpha-SMA is the marker for myofibroblasts, the cells primarily responsible for wound contraction.

**Kate Fitzgerald**

The dendritic architecture of a neuron determines how it receives inputs, and thus, changes in dendrite morphology will affect connectivity among neurons. Aberrant changes in the development of the arbor, or after the arbor has formed, can disrupt the functioning of neural circuits, causing severe brain dysfunction and leading to pathologies seen in cognitive disorders, neurological diseases, and trauma. In traumatic brain injury (TBI), decreased dendrite branching and other morphological abnormalities observed in the affected areas, result in functional deficits. Despite improved knowledge of the mechanisms of TBI, there is still no agreed-upon treatment for the secondary excitotoxic effects of the trauma. As promising drugs have failed in recent years, additional research is needed to re-examine models of TBI with the intent of finding a treatment that can protect injured neurons from long-term damage and death. In fact, surviving neurons of TBI-like injuries appear to express certain pro-survival factors, such as brain-derived neurotrophic factor (BDNF). Thus, the **objective** of this study is to analyze how changes in the dendritic arbor caused by TBI affect the functionality of neural networks and whether these changes can be prevented or rescued by BDNF. To assess changes in network dynamics, we use micro-electrode array (MEA) technology and calcium imaging, which respectively garner data with excellent temporal and spatial resolution. Our **hypothesis** is driven by the fact that calcium influx through synaptic N-methyl-D-aspartate receptors (NMDARs) promotes neuron survival by triggering cAMP response binding element (CREB) and BDNF expression, whereas calcium...
influx through *extrasynaptic* NMDARs results in the opposite. Additionally, we are motivated by previous work in our lab demonstrating that BDNF increases proximal branching in hippocampal neurons via a CREB-dependent mechanism. The *rationale* of this study is that if we can prevent additional damage *in vitro* caused by the excitotoxic effects of TBI, then these findings will inform future *in vivo* treatments. We will test our hypothesis through the following aims: 1) to determine whether BDNF can protect hippocampal neurons from morphology changes associated with excitotoxic damage (varicosity formation and cell death) and 2) to determine whether BDNF can prevent decreases in network-wide synchronization that is caused by sub-lethal glutamate treatment as measured by MEA technology and calcium imaging. Thus far, we have determined that global application of BDNF during periods of active branching increases synchronization of hippocampal cultures as measured by MEAs. We are now working to establish whether overexpression of BDNF has a similar effect. We will then move on to evaluating whether BDNF can be neuroprotective against excitotoxic damage.

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<th>Mehdi Ghodbane</th>
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<td>Spinal cord injury (SCI) affects 259,000 individuals in the United States resulting in greatly reduced quality of life, as well as a significant economic burden. At the moment, biomarkers capable of assessing either injury severity or recovery are not available. Biomarkers for SCI could be used to develop therapies in a laboratory setting as well as evaluate the efficacy of therapeutics in clinical trials. Cerebrospinal fluid (CSF), due to its contact with the central nervous system, represents an appropriate fluid to study SCI biomarkers. Studies have begun searching for biomarkers in humans, but these studies cannot be controlled. Rat models of SCI have been extensively utilized and may potentially elucidate both the temporal progression and mechanism of injury, as well as therapeutic options to control SCI damage. However, the small volume of rat CSF has prevented a thorough study of spinal CSF biomarkers following SCI. To obviate this limitation, we propose to develop a microfluidic immunoassay capable of measuring a panel of proteins in small volumes. With the microdevice, we will extract small volumes of CSF from rats following experimental SCI and identify protein expression changes following SCI as potential biomarkers. We propose to accomplish this goal through the following specific aims: 1) to design a microfluidic device for performing miniaturized immunoassays and develop a computational fluid dynamic (CFD) model of antigen-antibody binding; 2) to experimentally validate the CFD model, optimize its operating parameters and demonstrate a successful on-chip immunoassay; and 3) to utilize the device to study CSF cytokine profiles following SCI. At this point, we have completed the first two aims. A manuscript has been submitted to <em>Microfluidics and Nanofluidics</em> detailing the development and validation of the model as well as the optimization of assay parameters resulting in quantitation in as little as 1.35 μL of sample volume. We are in the process of expanding the device to facilitate a larger number of samples. Once this is complete, we will commence our in vivo studies of biochemical changes in CSF following SCI to identify potential biomarkers.</td>
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<th>Andrea Gray</th>
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<td>Mesenchymal stromal cells (MSCs) are adult stem cells that are attractive as a cellular therapeutic due to their non-immunogenicity and many beneficial effects achieved primarily via the secretion of a wide variety of molecules. Despite promising clinical trial results regarding the safety of administering single-cell suspensions of MSCs to human patients, demonstrating significant efficacy of these cells has been more difficult. The inefficient homing of these cells to target tissues and their rapid clearance necessitates the use of very large and sometimes repeated doses. Additionally, the beneficial effects of MSCs are not necessarily constitutive, and appear to be enhanced by external activating factors. The degree to which this may be happening <em>in vivo</em> may be suboptimal or insufficient, especially when only a small fraction of cells make it to the target tissue or when the amount of stimulatory factors in the microenvironment may be relatively low. To begin to address these limitations, our <em>objective</em> is to develop a systematic <em>in vitro</em> approach for elucidating and optimizing MSC activation by soluble factors in a cell-immobilizing delivery platform. We <em>hypothesize</em> that the efficacy of MSC therapies can be improved by exogenous activation for a distinct response(s) and by increasing MSC persistence. We plan to test our hypothesis through the pursuit of the following specific aims: 1) to investigate the activation of MSCs by a panel of soluble factors and the effects of these on MSC-mediated modulation of target cells; 2) to validate and further optimize results from the activation screen in a polymer-based delivery vehicle which prevents MSC migration/clearance;</td>
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and 3) to implement and analyze the effectiveness of pre-activated and immobilized MSCs in promoting recovery and healing after acute traumatic tissue damage. To date, we have completed the first aim and have generated preliminary results for the second and third aims.

**Kristina Hernandez**

Excitatory neurotransmission is dependent on proper dendritic arbor development, as well as synapse formation and maturation. Many neurodevelopmental disorders show improper neuronal morphology, such as a reduction in dendrite number or branching. Nitric oxide synthase 1 (neuronal) adaptor protein (NOS1AP), encoded by a schizophrenia susceptibility gene, is an intracellular factor that alters neuronal morphology and the composition of the postsynaptic density. Studies from the Firestein and Brzustowicz laboratories demonstrated that NOS1AP expression is upregulated in postmortem samples from the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia. The finding that NOS1AP expression is elevated in the DLPFC of patients with schizophrenia, which is the same brain region in patients with schizophrenia that displays shorter dendrites and a reduced dendritic arbor, prompted us to investigate whether NOS1AP can influence human dendrite development. To investigate the role that NOS1AP plays in human dendritic arbor development, we overexpressed the NOS1AP gene in differentiating human induced pluripotent stem cell-derived neurons and used Sholl analysis to assess dendritic complexity. Exogenous overexpression of NOS1AP resulted in a decrease of dendrite branching in human neurons at the developmental time point when both primary and secondary branching is actively occurring. A previous study in rat showed that NOS1AP is able to compete with postsynaptic density-95 (PSD-95) for binding to nNOS, suggesting that NOS1AP may sequester nNOS away from NMDA receptors. The decoupling of nNOS and NMDA receptors would prevent the activation of nNOS, and therefore, disrupt NMDA receptor-dependent arborization. We tested if NMDA receptor agonists can reduce NOS1AP protein levels and restore normal dendritogenesis in hiPSC-derived neurons. We found that NMDAR agonists, D-serine and GLYX-13, reduce NOS1AP mRNA and protein levels in hiPSC-derived neurons. Next, we tested if the NMDAR agonists can restore normal dendritogenesis in hiPSC-derived neurons. Results suggest that NMDAR agonists increase/rescue dendrite branching in human neurons with increased NOS1AP expression. Finally, to investigate the mechanism by which NOS1AP negatively alters dendrite branching we tested whether NOS1AP can alter actin polymerization, a process required for new branch points to occur. Through an immunoprecipitation experiment, we showed that NOS1AP is associated with filamentous actin (F-actin). Next, we found that increased levels of NOS1AP result in a decrease in F-actin, while total actin protein levels are unchanged. The results indicate that NOS1AP influences dendritic branching by altering actin cytoskeletal dynamics. In addition, our findings suggest that NMDAR agonists may be a potential novel treatment for patients with schizophrenia associated with specific risk alleles at small nucleotide polymorphisms in the NOS1AP gene.

**Jake Jacobs**

Long Terminal Repeat retrotransposable elements (LTR-RTs) are a large group of eukaryotic transposable elements characterized by flanking repeats in tandem orientation—the LTRs. The LTR of these elements contains the promoter and other sequences that recruit proteins involved in the expression, replication, silencing, organization, and stability of these elements. A successful transposable element must maximize its reproductive amplification without jeopardizing its host, and several characterized LTR-RTs appear to accomplish this through the selection of integration sites away from protein coding sequences. However, despite the high relatedness of all studied LTR-RTs, a universal mechanism that explains how these parasitic elements avoid coding sequences has not been established. Through alignment of previously published de novo integration sites of the LTR-RT Tf1 from the fission yeast *Schizosaccharomyces pombe*, we found a strong integration preference for integration events near the binding site of Sap1, a DNA-binding protein that controls the directionality of DNA replication by causing polar fork arrest. The goal of my thesis is to identify what factors at Sap1 binding sites are important to Tf1 recruitment. Several possibilities exist: (1) Tf1 integrates into fork barriers, (2) Sap1 tethers the cDNA to the host genome and brings the transposon in proximity to Sap1 binding sites in the host genome, or (3) a combination of both scenarios where Sap1 can tether but requires a stalled fork for integration. My research this semester has shown that Sap1 binding alone is not sufficient for Tf1 transposition and that fork blockage is also necessary. I am currently working to
Identify which factors in the stalled replisome are necessary for this effect, sequence de novo Tf1 insertion sites with high-throughput sequencing technologies in strains lacking these proteins, and lastly, identify S-phase as the moment of integration for LTR retrotransposons. We are also working to move our findings to a mammalian system because we hypothesize that mammalian retroelements may be using fork barriers as integration sites.

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<th>Jose James</th>
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<td>Food allergy is a common ailment with a prevalence that can be as high as 8% in children [1]. While a broad spectrum of individuals is allergic, the allergens to which patients typically react are a surprisingly small set of ingredients, primarily shellfish, peanuts or milk. Understanding why this small set has an increased risk for atopic reactions can improve predictions of allergenicity among food products and provide a deeper appreciation of the role of digestion in exposure of food antigens to the immune system. Focusing on crustaceans, the primary allergen in shrimp is tropomyosin (sTM). Using a peptide tiling array of sTM fragments on the sera of shrimp sensitive patients, 5 major IgE epitopes were discovered that consistently bound to IgE, indicating that there may be a sequence specific character within allergenic peptides [2]. Moreover, unlike TM found in crustaceans, orthologs in pig (pTM) and other vertebrates typically do not generate an atopic response. This suggests a divergence between invertebrate and vertebrate TM that affects the allergenicity of the protein. However, two main questions remain unresolved: (1) what differentiates sTP from its non-atopic vertebral orthologs, and (2) within the sTP sequence, what differentiates allergen epitopes from the remainder of the sequence? Therefore, the long term goal is to elucidate how protein sequence and the resulting structural characteristics, especially within the context of the GI tract and digestion, confer risk of an allergic response. Toward this goal, the current objective is to understand how the structure and stability of sTM affects the survival of epitopes for immune exposure. The central hypothesis of the proposed experiments is that the allergenicity of an epitope in sTM is dependent on the stability of the corresponding sequence which prevents digestion either from peptic or duodenal proteases, thereby allowing epitope exposure to immune cells distally in the GI tract. The hypothesis derives from the observation that thermal denaturation of sTM shows a clear and singular transition state that suggests global unfolding. Conversely denaturation of pTM exhibits two transition states signifying multiple shifts between folded and unfolded domains. Moreover, phylogenetic comparison between vertebrate (non-atopic) and invertebrate (atopic) sequences illustrates a divergence at position 126, in which vertebrates all have glycine and invertebrates do not, notably sTM has methionine. It was found that a Gly126 Ala mutation in rabbit skeletal TM enhances stability of the a-helix and creates a high MW intermediate during trypsin proteolysis [3]. Thus we suggest that Gly126 plays a crucial structural role in maintaining the global stability of sTM. Using TM as a model system for studying allergy at the protein structure level, we plan to test our hypothesis through pursuit of the following specific aims. (1) Resolve whether the set of sTM epitopes that survive gastrointestinal digestion contain sequences reported to be most-associated with food allergy. Our working hypothesis is that survival of digestion is a prerequisite for necessary immune exposure that would induce an allergic response, and therefore we expect an overlap between epitopes that survive digestion and those clinically found to be associated with allergy. (2) Determine whether a Gly residue at position 126 is correlated with decreased thermal stability and resistance to proteolytic cleavage. Our working hypothesis is that a Met126Gly substitution in sTM will create a protein that behaves more like pTM, with multiple transition states on thermal denaturation and increased susceptibility to digestion. Conversely, similar to the findings reported for rabbit TM, a Gly126 Met substitution in pTM is predicted to create a protein with enhanced stability and decreased proteolytic susceptibility.</td>
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<th>Paulina Krzyszczek</th>
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<td>Non-healing, chronic wounds affect 6.5 million people in the United States. Current treatments focus on wound management through debridement and frequent dressing changes, instead of promoting healing mechanisms. As a result, wounds remain open, which leaves them highly susceptible to infection. Uncontrolled infection can damage the underlying bone and muscle tissue, eventually requiring amputation. An approach that efficiently delivers factors that promote wound healing would close the wound faster and decrease the risk of infection and amputation. One protein that has been shown to play a role in wound healing is insulin. In previous animal studies and clinical trials, topically applied insulin resulted in the</td>
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complete healing of diabetic ulcers, which account for almost 25% of all chronic wounds. Since diabetics produce dysfunctional insulin (type II diabetes) or do not produce it at all (type I diabetes), it is not surprising that an insulin treatment demonstrates beneficial results. Similarly, following burn injury, victims do not respond to insulin to the same extent as they normally would, so a treatment that provides enough insulin to overcome this resistance may also be beneficial for burn wounds. A limitation with previous insulin studies is the use of frequent doses that are not responsive to local factors in the wound environment. In addition to insulin, wounds respond to many other cytokines and growth factors to progress through the phases of wound healing. For instance, mesenchymal stem cells (MSCs) secrete vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and platelet derived growth factor (PDGF-BB), along with many other key ingredients necessary in wound healing. The use of MSCs for the treatment of chronic, surgical, and diabetic wounds has demonstrated accelerated healing in clinical trials. A problem with MSC delivery to wounds in current studies is low cell survival and therefore, growth factor delivery to the wound is not maximized. Ultimately, there is a great need for a treatment that can provide a sustained release of insulin and other factors to stimulate healing of problematic wounds. The objective of this study is to develop a topically applied dual-cell therapy consisting of insulin-producing porcine pancreatic beta cells and human MSCs to accelerate healing of diabetic ulcers and burn wounds. This proposed solution involves protecting cells from immune attack by microencapsulation using the polymer poly(ethylene glycol) diacrylate (PEGDA), which is FDA-approved, non-immunogenic, and can be functionalized to become biodegradable. The central hypothesis is that topical application of this dual-cell therapy will prolong the release of insulin and growth factors directly to the wound site. Insulin is glucose-responsive so beta cells will deliver it based on the glucose levels in the wound or delivery environment. Secreted factors from both cell types will result in accelerated healing of diabetic ulcers and burn wounds. Cell-cell contact and paracrine signaling can be maintained within the microspheres, resulting in high cell viability and a sustained release of secreted factors tailored to the specific wound microenvironment. The rationale of this study is that topically applied insulin and MSCs have separately shown promise in previous wound healing research, but can be greatly improved upon with a combinatorial approach that uses microencapsulation. This study will be guided by the following aims: 1) to optimize dual-cell encapsulation parameters and schemes based on cell viability; 2) to maximize levels of secreted factors using different dual-cell encapsulation parameters and schemes; 3) to evaluate the effectiveness of the dual-cell therapy using an in vivo porcine wound model.

Narendra Kuber

Late Infantile Neuronal Cereoid Lipofuscinosis (LINCL) is a lysosome storage disorder caused by the deficiency of a lysosomal protease, Tri-Peptidyl Peptidase I (TPP1). LINCL belongs to a group of related hereditary neurodegenerative diseases known as Neuronal Cereoid Lipofuscinosis (NCL) that occur at a frequency of 2 to 4 in 100,000 live births. LINCL shares a common causality with other prevalent lysosomal storage disorders that also result from specific lysosomal enzyme deficiencies. In LINCL, TPP1 deficiency results in accumulation of a substrate that is normally degraded, resulting in cell death. Neurons and photoreceptors are the primary cells affected. LINCL has a poor prognosis and currently there is no cure for this fatal disease. A breakthrough in treatment for LINCL will provide a template for development of therapies for similar lysosomal disorders. One approach to treatment for LINCL is gene therapy, in which targeted cells serve as factories that secrete TPP1 for utilization by other surrounding cells deficient in the enzyme. This approach is subject to concerns about mutagenesis of transformed cells and an inability to stop treatment in the event of an adverse reaction. Another approach is enzyme replacement therapy (ERT). This approach is attractive because of flexibility in dosing and an ability to stop treatment if necessary. ERT can be carried out using three delivery routes: intravenous (IV), intrathecal (IT) and intraventricular. Currently, IT and IV injection method is being used to deliver TPP1 in LINCL mice. IT method overcomes the blood brain barrier (BBB) while IV method is faced with this hurdle. Novel delivery methods using IV injection are being developed that will help the protein to overcome the BBB. Regardless of which method is being employed, it is advantageous for TPP1 to reside longer in the lysosomal compartment of the neurons. A TPP1 variant with a long half-life will reduce the therapeutic quantity of recombinant TPP1 required by reducing the dose and the frequency of administration. The objective in this
study is to engineer a TPP1 variant with a long intra-lysosomal half-life with improved efficacy. Such a variant will be a potential drug candidate to treat LINCL. We hypothesize that stabilizing TPP1 structure by substitution of one or more amino acids will result in a variant with an increased intra-lysosomal half-life that will increase survival of neurons by reducing and maintaining a reduced amount of accumulated substrate in their lysosomes. Preliminary studies of ERT in TPP1-deficient mice reveal that IT administration of recombinant human TPP1 results in levels of TPP1 in brain higher than those in wild-type mice. This TPP1 activity eliminating further accumulation of substrate but has little effect on reducing levels of already accumulated substrate. The reason for this could be that injected TPP1 is not available at the same concentration to all neurons in the brain because some neurons are deeply situated. The rationale of this study is that a TPP1 variant with a longer half-life will be effective at the lower concentration that is available to inner neurons. We hypothesize that the longer-lived protein will be capable of maintaining reduced levels of substrate and decreasing levels of previously accumulated substrate essential to reversing symptoms. We plan to test this hypothesis through pursuit of the following specific aims: 1) design TPP1 variants using computational methods and assess TPP1 activities in vitro; 2) compare half-lives of active variants to that of wild-type TPP1; 3) perform in vivo studies to determine if active variants with longer half-lives are more effective than wild-type TPP1 in prolonging survival of LINCL mice and 4) conduct histopathological analysis of tissues in various organs such as heart, brain, kidney and liver in treated LINCL mice to determine the extent of the lowered burden and compare this ability to that of wild-type TPP1. These studies will provide the foundation for a treatment to improve the quality of life of LINCL patients.

### Serom Lee

Allergic contact dermatitis is an inflammatory skin disease that is a rising public health concern due to its growing prevalence. To assess the public’s propensity for contact dermatitis, many alternatives to in vivo screening of chemicals have been developed. However, these systems are limited by their poor ability to accurately identify a subset of contact sensitizers known as pro-haptens that require metabolic activation to serve as the antigen. Furthermore, current in vitro systems are restricted to evaluating a small panel of biomarkers of sensitization that are not sufficiently predictive. To address these limitations, our objective is to develop an in vitro/in silico alternative to animal testing for screening pro-hapten skin sensitizers and to identify molecular patterns that are predictive of sensitization. The rationale of this study is that an integrated source of metabolism such as the use of human liver microsomes as a source of cytochrome p450 enzymes will metabolize pro-hapten sensitizers to form reactive products that will activate antigen presenting cells (APC) such as Mutz-3 derived Langerhans cells. A variety of in vitro metrics can be subsequently evaluated to expand the panel of biomarkers and identify a more predictive molecular pattern. To aid in the discovery of a sensitization signature, computational tools such as a support vector machine can be utilized. This is a powerful machine learning algorithm that will 1) perform feature selection to identify the best biomarkers of sensitization and 2) act as a classification model to predict the sensitization potential of chemicals based on the key metrics identified. This type of in vitro/in silico integrative approach can eventually replace cosmetic animal testing and can also be used to further examine the molecular and cellular events that occur during skin allergy. To date, we have completed the development of the computational model using the support vector machine and identified a molecular signature that includes IL-12, IL-9, VEGF, and IFN-γ using pro-hapten isoeugenol and p-phenylenediamine in a pilot study using a co-culture with a skin equivalent and Mutz-3 derived Langerhans cells. We are currently in the process expanding our chemical panel of pro-hapten sensitizers to validate the utility of the identified cytokine metrics with our microsome activation system.

### Trevan Locke

Cases of advanced solid cancer (e.g. sarcomas, carcinomas, and lymphomas) 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 specifically 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 using 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. Preliminary findings have established that
GALA conjugated to lipid and embedded in a liposome forms an alpha helix when exposed to acidic conditions and triggers
pH-dependent release from egg- phosphatidylcholine:cholesterol liposomes acting as an endosome analog. 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 plan to test our hypothesis
through the following specific aims: 1) to engineer liposomes containing anti-PSMA ligands and GALA in order to investigate
the conditions in which the corresponding functionalities operate effectively; 2) to show that anti-PSMA fusogenic
liposomes loaded with doxorubicin exhibit a) selective targeting of tumor endothelium analogs, b) effective release of
doxorubicin, and 3) enhanced killing of targeted cells.

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| Blood vessel assembly is a conserved process that involves the formation of a lumen, or central tube. Understanding the
mechanism by which this process occurs is critical in developing therapeutic targets for a variety of pathologies, including
congenital heart defects and tumor angiogenesis. In the fruit fly *Drosophila melanogaster*, dorsal vessel formation involves
the migration of progenitor cells, called cardioblasts (CBs), along the dorsal midline. CBs eventually move toward each other
and undergo morphological changes to form a linear tube. Opposing CBs first establish contact and attachment at their
dorsal lumenal surfaces. They then attach at their ventral surfaces, while simultaneously being repelled in between,
allowing for the formation of a lumen. The Slit protein and its corresponding receptor, Roundabout (Robo), facilitate
repulsion of opposing CB membranes by negatively regulating E-Cadherin mediated cell adhesion between these surfaces.
However, the concomitant mechanism by which attraction and subsequent formation of adhesion domains are facilitated
remains to be understood. The objective of this study is to gain insight into the coordinated regulation of signaling proteins
in promoting the discrete cell shape changes leading up to the formation of a complete biological tube. The central
hypothesis of this study is that the Netrin-Frazzled signaling pathway, which was previously characterized as a mediator of
axonal crossing and attraction in the developing *Drosophila* CNS, promotes CB attraction and attachment in a site-specific
fashion in conjunction with Slit-Robo signaling. We base our hypothesis on the following observations: 1) the guidance
molecule Netrin and its corresponding receptor Frazzled have been known to act in opposition to Slit-Robo signaling in the
CNS, allowing for coordinated attraction and repulsion of axonal growth cones, and 2) frazzled loss-of-function mutants
display attachment defects at sites of cell-cell adhesion, and a similar phenotype occurs in netrin deletion mutants.
However, unlike the paracrine signaling mechanism displayed by the CNS, we have determined via fluorescence in situ
hybridization (FISH) that CBs produce both Netrin and Slit and their corresponding receptors. As such, the rationale of
the study is that if we can elucidate the mechanism by which the coordinated expression and localization of Netrin and
Frazzled in the heart is regulated, we can form a more cohesive picture of dorsal vessel morphogenesis by comparing and
analyzing any interactions with the Slit-Robo signaling pathway. We plan to test our hypothesis through the pursuit of the
following specific aims: 1) determine what regulates the differential expression pattern displayed by netrin in the dorsal
vessel via genetic and molecular biology techniques; 2) determine the morphological consequences of ablating Netrin and
Frazzled function in the dorsal vessel via live-imaging of embryos, and 3) determine the effect of overexpression and
misexpression of netrin and frazzled in the dorsal vessel and surrounding tissues, in both a wild-type background and a
**Ileana Marrero-Berrios**

There are approximately 12,000 new cases of spinal cord injury (SCI) annually in the US and currently ~270,000 people in the US are living with SCI. SCI is characterized by an initial mechanical injury, which is followed by a cascade of secondary events, including chronic inflammation, leading to progressive destruction of spinal cord tissue. Many therapies are being developed to treat the early events in SCI, including cell therapy using mesenchymal stem cells (MSCs) derived from bone marrow. MSCs promote therapeutic benefits via secretion of soluble cues which control immune cell functions and provide trophic support, hence could potentially reduce the acute macrophage inflammatory response following tissue injury and promote recovery. MSCs can be encapsulated in an alginate matrix, where they are immobilized and can be surgically implanted at traumatic injury sites. To date, however, the effect of local anesthesia on the encapsulated MSCs function has not been evaluated. Recent evidence suggests that MSCs are sensitive to the antiproliferative effects of local anesthetics at concentrations of 10-100 μM. These anesthetics do not induce cell death, but inhibit cell growth by impairing cellular mechanisms that promote cell-to-cell communication and inhibit mitochondrial respiration. The objective of this study is to determine whether anesthesia has an effect on the anti-inflammatory properties of encapsulated MSCs. We will test the effect of increasing concentrations of three anesthetics: Lidocaine, Ropivacaine, and Procainamide which will be included in the MSCs culture. Treated and untreated MSC secretions will be evaluated by quantifying IL-6 and PGE₂ using ELISA. Cell viability will be evaluated by imaging techniques with calcein and ethidium homodimer. As well, cell proliferation/ viability will be assessed by alamarBlue® assay.

**Adriana Martin**

An evolving set of compounds referred to as amphiphilic macromolecules (AMs) possess structures comprised of a sugar acid backbone (e.g. mucic acid) with attached aliphatic chains and a poly-ethylene glycol (PEGylated) linear hydrophilic tail. The conjugation of a PEG tail to the hydrophobic core gives the AMs its amphiphilicity, which improves solubility and allows for spontaneous self-assembled micellar formation in aqueous solutions. Given these properties investigators are considering AMs for applications such as drug encapsulation in micelles or nanoparticle formulations and as a liposome stabilization surfactant-like additive. It has been observed in previous studies that subtle changes to the core which measures at approximately one-tenth of the overall molecular weight, affects the behavior of the AMs in terms of cellular uptake via macrophages. However, to date no studies have been performed which examine in detail the relationship between AM structural characteristics and its ability to bind to biological membranes. The objective of this study is to expand the current understanding of molecular interactions between AMs and membranes. The central hypothesis is that the rate and propensity for membrane retention relies on the structural properties of the AMs as an important measure for facilitating the cellular uptake and bioactivity of the AMs. Our studies are focused on: 1) assessing the prediction capabilities of a computational modeling approach for measuring the effects of structural changes to AMs on membrane interactions, and 2) using information acquired experimentally to validate the theoretical simulations and understand AM properties further. The aims of this project are as follows: 1) to characterize the interactions of AMs with a simulated lipid bilayer using computational modeling; 2) to experimentally determine and validate simulations of the AM-bilayer interaction using quartz crystal microbalance with dissipation techniques (QCM-D) with a model lipid bilayer; and 3) to observe the effects of AM dosing on cellular membrane behavior and uptake in vitro. The propensity of the AM series to interact with a membrane biomimetic system was investigated using coarse-grained molecular dynamics (CG-MD) and experimental binding studies via Quartz crystal microbalance with dissipation (QCM-D). In agreement, both the computational and experimental results reveal that AM cores with variations of charge, hydrophobicity and stereochemistry resulted in the classification of high versus low binders based on penetration distance, mass deposition and kinetic measurements of membrane binding. A rank list was established with charged structures with a more amphiphilic nature (determined by hydrophobic:hydrophilic assessment) binding better than structures without a charge and/or without a hydrophobic backbone. This determination of influencing factors on membrane binding can be used as a reference for future AM synthesis. More broadly, this study affirms that our integrated computational-experimental approach provides a robust strategy for a guided design of AMs for...
therapeutic applications. This project’s insights and results to date have been compiled into a penultimate manuscript draft entitled: *Biophysical characterization of the interaction between amphiphilic macromolecules (AM) and lipid bilayers: modeling the role of AM core structure and composition.*

**Ilija Melentijevic**

Understanding endogenous mechanisms for neuronal protection is critical for the effective design of any intervention that protects the nervous system from age-associated decline. To define neuroprotective approaches operative in a living organism, we are studying a simple model in which we can directly observe individual labeled neurons in the transparent nematode *C. elegans*. We have documented how some of these neurons age, finding striking similarities to human brain aging – little neuron loss, morphological restructuring, and synaptic degeneration. Recently we noticed a new and previously unknown feature of young adult neurons – neurons can exude substantial packets of cellular contents (which we termed exophers) that can include aggregated human neurodegenerative disease proteins or mitochondria. The ability to dump cell contents appears to decline later in adult life. The objective of this study is to understand the function of the exopher. The central hypothesis of this study is that the neuronal extrusion phenomenon constitutes a significant pathway by which healthy neurons maintain their functions. This hypothesis is based on the finding of aggregation prone proteins inside the exopher. We plan to test our hypothesis through the pursuit of the following specific aims: 1) establish the relationship between exophers and autophagy, test a mechanism for their removal after extrusion; 2) determine whether specific neuronal stresses lead to increased exopher production; and 3) identify genetic factors that increase or decrease exopher production using genetic screens.

**Sarah Misenko**

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, 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 test genetic interactions between specific genes of the mammalian DSB response network. A particular gene of interest is Bloom’s helicase (*BLM*). Mutations in the *BLM* gene cause predisposition to tumor development, developmental abnormalities, and chromosomal instability. It has been suggested that *BLM* is required for HR, specifically in the resolution of Holliday junctions and DNA resection. There is currently no cure for *BLM*-deficient patients and a better understanding of the function of *BLM* may lead to novel treatment options. Our central hypothesis of this study is that *BLM* functions to disassociate RAD51 loading on single-stranded DNA at replication forks and after DNA resection, aiding in proper replication and DNA DSB repair regulation. We plan to test this hypothesis through the following specific aims: 1) determine if *BLM* plays a role in the disassociation of RAD51 on single-stranded DNA by quantitating RAD51 foci in *BLM* deficient B cells; 2) determine if *BLM* deficient cells are hypersensitive to replication stress by measuring damage response signals and genomic instability after replication stress induction; and 3) determine if reducing the RAD51 loading in *BLM* deficient cells can rescue the genomic instability by analyzing aberrations in metaphases after loading interactions are disrupted.

**Daniel Myers**

The Biopharmaceutics Classification System (BCS) characterizes oral drug products into one of four classes based on their water solubility and membrane permeability. BCS class IV drugs exhibit both low solubility and low permeability which leads to very poor bioavailability. As a result, only a few BCS class IV drugs have made it to market. There is a need for an effective delivery platform that can overcome the undesirable properties that limit the potential of Class IV drugs given that many of the new molecular entities being developed today are class IV. The main limitations for oral drug delivery include poor stability in the GI tract environment, low solubility, and inadequate permeability through the intestinal membrane. Encapsulation in polymeric nanoparticles (NPs) provides a drug delivery vehicle suitable to overcome these limitations. Flash NanoPrecipitation (FNP) using a multi-inlet vortex mixer is a recently developed method to encapsulate hydrophobic drugs in stable block copolymer NPs with a narrow size distribution. In order to target the GI tract and improve the transport of NPs across the intestinal epithelium, a peptide ligand can be conjugated to the NP surface using copper-free click chemistry (CFCC). To select an optimal targeting ligand, we have screened peptide libraries against Caco-2 gut epithelial...
monolayers using phage display to assess Caco-2 uptake. The **objective** of this study is to formulate a NP platform to improve the bioavailability of BCS class IV drugs by overcoming poor solubility and inadequate intestinal permeability. FNP will be used as a tunable method to encapsulate hydrophobic drugs, and a novel peptide ligand conjugated to the NP surface will function as both a targeting agent and a promoter of intestinal uptake. Three peptides have been isolated in a lipid-dependent manner using T7 phage display, with the best peptide (P19) showing 100,000-fold greater uptake compared to control sequences. We hypothesize that the conjugation of a selected phage display peptide to the surface of block copolymer NPs containing drug will result in increased binding and uptake across the intestinal epithelial monolayer and, ultimately, provide a platform to improve the oral bioavailability of BCS class IV drugs. Our **rationale** is that the conjugated peptide will enable NPs to overcome the barrier of the intestinal epithelium and adequately deliver drug to the underlying lymphatic and blood vessels. This work will be carried out in the following aims: 1) Design, characterize, and optimize peptide-conjugated polystyrene-poly(ethylene glycol) (PS-PEG) block copolymer NPs using FNP to obtain a narrow size distribution of stable NPs with high encapsulation efficiency and sufficient drug loading for multiple class IV drugs. We have determined the optimal polymer:core ratio for the formation of monodisperse PS-PEG block copolymer NPs with a Vitamin E core. We found that the encapsulation of ritonavir (RTV), an HIV protease inhibitor, is dependent on the presence of free Vitamin E as a more hydrophobic entity at ≥60 mol% of the NP core. We have evaluated the effects of polymer concentration, Vitamin E:RTV ratio in the core, and dilution of the mixer outlet stream on the size, polydispersity, and RTV encapsulation efficiency of PS-PEG NPs. We have synthesized P19 and a control sequence and conjugated them to the surface of PS-PEG NPs containing RTV in the core. 2) Assess the in vitro transport of phage display peptides, including P19, and peptide-conjugated NPs across a Caco-2 cell epithelial monolayer to determine the extent of drug uptake. In a pilot study, we performed transport experiments across Caco-2 monolayers for NPs displaying P19 and a control peptide with a vitamin E core. The control peptide was designed to disrupt a postulated salt-bridge critical for amphiphilicity. The study showed that both free P19 peptide and the NP displaying P19 are transported in a lipid-dependent manner while the control peptide and control NP are not. Moreover, the transport rate of the P19 NP was four-fold higher than the transport rate of the control NP, demonstrating the importance of the salt-bridge for oral absorption. 3) Evaluate the in vivo oral bioavailability of NP-encapsulated class IV drugs using a rat intestinal perfusion model.

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<td>Orthotopic liver transplantation is the only available therapeutic option for end-stage liver disease, but is limited by the scarcity of suitable livers resulting in about 4,000 annual deaths of transplantation candidates in the US alone. Abnormally high hepatic level of triglycerides (TG) in the form large fat droplets, known as macrosteatosis, is the most common single predisposing risk factor for postoperative liver failure after transplantation leading to the discarding of ~1,000 macrosteatotic livers annually. Thus, techniques to salvage such livers would ameliorate the current donor liver shortage. Reduction of macrosteatosis by dieting for several days to weeks prior to live donor liver transplantation in humans and in animal models has led to successful liver transplantation. However, in the context of using brain dead liver donors, organ procurement and transplantation is typically done in a 12 hour timeframe and therefore macrosteatosis reduction must be accelerated. The central hypothesis of this project is that discarded macrosteatotic donor livers can be salvaged within the clinically relevant timeframe of a few hours by ex-vivo machine perfusion with an optimized cocktail of agents that stimulate the metabolism and removal of fat stored in liver, thus reducing the risk of postoperative liver dysfunction. To optimize such a defatting cocktail, a novel in-vitro system of macrosteatosis using primary adult rat hepatocytes was developed [2]. Culturing lean hepatocytes in the presence of high levels of free fatty acids promoted fat accumulation in the form of macrosteatotic lipid droplets with volumetric characteristics similar to those found in human macrosteatotic livers. Using this system, we found that supplementation of previously identified defatting agents accelerated lipid droplet breakup 2-fold, eventually reaching the level of lean cells [2]. Ultimately, successful transplantation will be contingent upon the conservation of critical hepatocyte functions. We have previously observed that macrosteatosis decreases urea secretion and bile canalicular transport. This functional impairment was reversed by accelerated defatting, without compromising...</td>
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viability, as the resulting urea secretion rates and bile canalicular transport were comparable to lean hepatocytes used as a positive control [2]. This model is ideally suited to further explore the effect of defatting approaches on macrosteatosis levels as well as hepatocyte viability and function. I am continuing to characterize the defatting effect of other cocktails in this manner and am including an even broader spectrum of hepatocyte specific functions, such as albumin and fibrinogen secretion. In addition, I am evaluating the ability of such rapid defatting to reduce the hypersensitivity of macrosteatotic hepatocytes to oxidative stresses that accompany liver transplantation using an ischemia/reoxygenation in-vitro injury model developed in our lab. Finally, together with Dr. James Guerrera at Columbia University Medical Center, NY, I am initiating a new study in which discarded human macrosteatotic livers will be perfused using our defatting cocktail to investigate the process of macrosteatosis reduction and its effect on human liver function and transplantability. The New York Organ Donor Network has agreed to provide livers for this breakthrough study, which, if successful, will provide initial feasibility data in humans. In the short term, translation of this new technology is expected to add 1000 livers per year to the donor pool, which will potentially reduce deaths by the same number among patients waiting for donor liver grafts.

### Antoinette Nelson

Approximately 69% of HIV infected individuals are found solely within the Sub-Saharan region of Africa. Without the development of an effective cure, it is believed that the most promising way to slow down the spread of HIV/AIDS in South Africa is through widespread education and the empowerment of women within rural communities. One critical means of empowerment is by providing access to pre-exposure prophylaxis (PrEP) treatments, for the prevention of viral infection, which is within patient control. This has led to the development of microbicides, which are topical therapeutic agents that may be applied vaginally or anally prior to intercourse, to prevent HIV transmission through sexual contact. Efforts to suppress HIV infection through the use of these prophylactic therapies, has yielded remarkable results. However, a major limitation of PrEP agents is that patient adherence is typically low and the primary cause of ineffective treatment, ultimately leading to HIV infection. One of the main reasons for the lack of compliance is that current available formulations are not well received due to leakiness, messiness, and the requirement of administration too close to the time of intercourse. Thus, there exists the need for a PrEP therapy that induces minimal leakage from the application site and allows for earlier and less frequent administration to improve comfort and patient compliance. It is also important for our any therapeutic to be efficiently taken by the intestinal tissue, another obstacle faced by present therapies. Our lab has developed a foam formulation capable of delivering therapeutic agents to the gut mucosal region with limited leakage and timely foam breakdown to trigger drug release within the colorectal tissue. Also, through the use of phage display, we have identified a number of novel peptide sequences with the ability to increase carrier penetration into intestinal tissue. The objective of this study is to formulate and assess pharmaceutical foams that cover the gut mucosal surface in order to homogeneously distribute carriers capable of translocating across the colorectal mucus and delivering drugs in a sustained manner. The central hypothesis is that our foam formulations will maximize surface coverage within the colon, sustain low local 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 will be guided by the following aims: 1) 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; 2) 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; and 3) to design, optimize and evaluate nanocarrier capability to block HIV entry and cell-to-cell HIV transmission in order to reduce drug/peptide and nanocarrier doses.

### Jillian Nguyen

There is evidence that when the human brain transforms sensation and perception into action, the process involves the integration of “top-down” executive functions and “bottom-up” sensory inputs. The mechanisms that underlie this process are highly adaptive, allowing for the behavioral flexibility that is essential for interaction with dynamic and complex
environments. However, how top-down and bottom-up signals are selected, prioritized, and applied to mediate perception and action is not fully understood. Neuropsychological and electrophysiological studies provide evidence for anatomical and functional divisions in ventral and dorsal processing for sensory modalities such as vision, audition, and touch. However, the notion that the human brain translates, for example, visual information into distinct, dissociated pathways for perception and action is widely debated. Moreover, the neural correlates for high-level executive functions that mediate volition are poorly understood. The **objective** of this study is to elucidate the way in which top-down processes mediate human behavior. Our **central hypothesis** is that top-down processes, including perceptual schemas and volitional control, play a more significant role in motor behavior than has been documented in previous studies. We will pursue testing the hypothesis by focusing on implementing advanced techniques that rely on full measures of natural behavior to clarify existing issues regarding interactions between top-down and bottom-up processing. Our **rationale** is that with validation of these methods, we can then tailor our analyses to benefit clinical populations by gaining a better understanding of the mechanisms behind nervous system perturbations. The **specific aims** are (1) to clarify the role of top-down signaling on modulating sensory-motor behavior, and (2) to identify human brain regions involved in mediating volitional control of these processes. We first assess the selection and recruitment of top-down and bottom-up processes by characterizing the influence of top-down signals on motor action when these two modalities compete. We study the unfolding of reach behavior in the context of intended, goal-directed actions as well as spontaneous, automatic movements, using a robust 3D depth-inversion illusion (DII) to track sensory-motor changes that may occur. Since past studies only focused on deliberate, goal-directed actions, we provide an innovative approach to assess the full motor loop to clarify long-debated issues in ventral and dorsal stream information processing. In addition, we propose the implementation of biofeedback-EEG training to localize brain regions associated with top-down executive function during a simple motor imagery task. Unlike conventional brain-machine interfaces (BMIs) that pre-define activation regions, we use a Bayesian-sparse-probit classifying algorithm to automatically uncover regional activity during volitional control of a directional task. This paradigm can potentially identify neural correlates of intentionality that shape the formation and execution of one’s actions. By investigating natural behavior using objective and automatic measures, we provide powerful analytical tools that improve on existing methods to help expand the field of neuroscience.

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| Mutations in the *blm* gene can cause Bloom’s Syndrome, a genetic condition associated with cancer predisposition and developmental abnormalities for which there is presently no cure. Homozygous mutation of *blm* in mice has been demonstrated to cause genomic instability, and this has been attributed to a requirement for BLM in the homologous recombination (HR) DNA repair pathway. Specifically, BLM has been suggested to be required for disassembly of the Holliday junction, a DNA intermediate structure in the HR pathway. In recent years, a new function has been suggested for BLM by analogy with a yeast gene, *sps1*, which is hypothesized to be homologous to mammalian BLM. *In vitro* biochemical evidence suggests that BLM is involved in resection at sites of DNA double-strand breaks (DSBs), potentially acting with an exonuclease, Exo1, as the regulatory step to begin HR. An involvement in DSB resection would represent a second function of BLM in the HR pathway. BRCA1 plays a critical role in promoting HR mediated repair of DSB. Recent studies have demonstrated that defects in HR in BRCA1 deficient cells can be corrected by a loss of 53BP1, a protein that promotes an error-prone DSB repair pathway, termed non-homologous end joining (NHEJ). Presently, there is limited understanding of the specific role of BLM in maintaining genomic stability and its interaction with proteins involved in HR and NHEJ to promote faithful repair of DSBs in mammalian cells. The **objective** of this study is to understand the specific function of BLM in DNA resection, which may enable the development of novel treatment options for cancers that arise from genomic instability, particularly BRCA1 mutations. The **rationale** of this study rests with the finding that loss of 53BP1 is able to reverse the DSB repair defect phenotype associated with BRCA1 loss, termed synthetic viability. Because the proposed role of BLM is the regulation of HR via DNA resection similar to BRCA1, we **hypothesize** that a defect in HR in BLM deficient mammalian cells can be compensated by depletion of 53BP1. We propose the following **specific aims** to test the
**William Pfaff**

Meniscal lesions are one of the most common intra-articular knee injuries in the United States. The meniscus plays a role in load bearing, shock absorption, stability, and lubrication of the knee, and any damage to the meniscus can cause joint pain and discomfort. Injuries or tears to the meniscus can eventually lead to the deterioration of the intra-articular cartilage and lead to osteoarthritis of the knee joint. Currently the most common treatment for meniscal lesions is removal of the damaged tissue via meniscectomy. However, by removing the meniscal tissue the knee loses its mechanical properties, and there is a positive correlation between meniscectomies and long-term degradation of the knee. There is a need for a functional meniscus implant that provides a scaffold that offering mechanical stability as well as a scaffold for cells to penetrate and regenerate fibrocartilage. For our study a meniscal implant was constructed by hand-weaving a continuous poly(DTD DD) fibers circumferentially around a wedge pattern, creating a 3-dimensional scaffold resembling a meniscus with two elongated tails for solid fixation into bone channels. The polymer scaffold was then cross-linked with EDC and injected with type I bovine collagen and sterilized with gamma radiation at 25 kGy. A sheep model is used to test the efficacy of these implants. The objective of this study is to observe the efficacy of these implants by performing meniscectomies on the control group of sheep, and insert meniscal implants on a second population of sheep. These sheep will be sacrificed after several months to observe how the implant reduces degradation of the intra-articular cartilage of the knee. The central hypothesis of this study is that if you insert the poly(DTD DD) fibers with injected collagen and fixate it onto the intra-articular site, the implant will be able to provide mechanical stability to the joint and prevent degeneration of the articular cartilage. The rationale of this study is that if a meniscus implant can successfully mimic the behavior of the native tissue and provide a site for regeneration then the implant will become a significant improvement over current meniscectomy treatments.

**Melissa Pryzborowski**

Diabetic foot ulcers (DFUs) occur in up to 10% of diabetics; they are painful and decrease quality of life. Currently, these ulcers account for 80,000 amputations each year, and, as the aging population grows, this incidence will increase. Many treatments have been developed for non-healing wounds, including dermal skin scaffolds and topical growth factors. Dermal scaffolds have had success in improving closure in DFUs over traditional wound care by providing a template for cell migration and vascularization; however, they do not address the complex inflammatory state of the wound tissue, which includes cellular dysfunction, decreased growth factor production, and high amounts of matrix metalloproteinases (MMPs). The RAGE (receptor for advanced glycation end products [AGEs]) pathway is implicated in this harsh wound environment. It stimulates a pro-inflammatory signaling cascade resulting in an influx of inflammatory cells, and subsequently, the MMPs. Therefore, when growth factors are added topically, they face failed cellular responses and degradation, which limits their success. The goal of this project is to develop a multi-modal treatment that improves dermal skin scaffolds by adding growth factors packaged specifically to survive the DFU milieu. The growth factor selected is stromal cell-derived-factor-1 (SDF-1), a chemokine that attracts stem and progenitor cells to the wound, improves vascularization, and upregulates other depleted growth factors. While SDF-1 provides dramatic improvement in wounds in healthy animals, preliminary results show no benefit in diabetic animals. Therefore, our hypothesis is that 1) SDF-1 action will be improved by attenuating the inflammatory response with the RAGE blocking agent soluble RAGE (sRAGE) and 2) SDF-1 persistence will be improved by packing SDF-1 into liposomes, leading to faster wound closure. sRAGE has previously been shown to block RAGE and immunomodulate the wound environment as well as increase growth factor production. To test this we will characterize the role of AGE-RAGE signaling on impaired SDF-1 action and restore function in vitro using sRAGE. We have found that the
sRAGE modulates superoxide production, which, in turn, improves SDF-1 function. Liposomes increase the half-life of a molecule and can self-assemble on the nanoscale. We will develop SDF-1 liposomes and characterize their activity in vitro. Ultimately, the soluble RAGE and SDF-1 nanoparticle treatment strategies will be combined in skin scaffolds and applied in an in vivo murine diabetic wound model. We have found that SDF-1 liposomes maintain SDF-1 function and improve wound healing, and are currently planning experiments for the combined treatment.

Ana Rodriguez

Proper synaptic transmission is essential for normal brain function and requires the precise spatial and functional assembly of signal transduction machinery at synaptic sites as well as correct patterning of dendrites and their branches. The postsynaptic density (PSD), an electron-dense region that characterizes the membranes of postsynaptic neurons, is a dynamic and complex structure. The PSD is composed of scaffolding and cytoskeletal proteins that localize receptors, ion channels, and signaling molecules at the synapse. A major scaffolding molecule found at the PSD of excitatory glutamatergic synapses is the postsynaptic density protein 95 (PSD-95). PSD-95 contains three N-terminal PDZ protein interaction domains (first discovered in PSD-95/Dlg/ZO-1 proteins) and is found in virtually every excitatory glutamatergic synapse, where it has been implicated in the trafficking and stabilization of glutamate receptors. It is widely accepted that changes in synaptic efficacy can result from alteration of excitatory neurotransmission mediated by postsynaptic glutamate receptors. Consequently, molecules that interact with these receptors and modulate their function – directly or indirectly – will have a significant effect on synaptic efficacy. Cypin (cytosolic PSD-95 interactor) was first identified as a protein that interacts with PSD-95 and disrupts its clustering at the synapse. Moreover, extensive research in our laboratory has identified cypin as a major regulator of dendritic branching in primary rat hippocampal neurons. When cypin is overexpressed at a time when dendrite branching is occurring, it promotes microtubule polymerization, resulting in a significant increase in both primary and secondary dendrites. Cypin has a clear role in the development of dendrite branches, and it is known to bind to PSD-95, but at this point its function in regulating synaptic dynamics is unclear. The objective of this study is to investigate the impact that altering cypin levels has on the structure of synaptic sites and neuronal signaling. Numerous studies demonstrate that PSD-95 may specifically mediate the synaptic localization and activity of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPA). Because the strength of synaptic transmission is thought to be largely dependent on the amount of AMPARs anchored at the PSD, it is essential to understand the consequences that interactions between PSD-95 and associated proteins that affect its location, such as cypin, might have during this process. Our preliminary data show that when cypin is overexpressed, total PSD-95 protein levels increase while PSD-95 synaptic targeting is disrupted. We hypothesize that by altering cypin levels in hippocampal neurons, the degree of AMPAR-mediated synaptic transmission will be affected, thus modulating the overall dynamics of neuronal networks. We believe that as PSD-95 is not trafficked to synaptic sites, and consequently the membrane when cypin is overexpressed, PSD-95 becomes more soluble and its distribution is altered. We will use biochemistry and cell biology techniques to identify molecular changes in the postsynaptic structure of hippocampal neurons when cypin levels are altered. Our preliminary results from subcellular fractionation studies show that when cypin is overexpressed, PSD-95 levels are reduced in the heavy membranes fraction of synaptosomes. Knocking down cypin results in an increase in PSD-95 expression in the same fraction. Moreover, cypin overexpression results in a decrease in dendritic spine density, which is associated with less mature spines. We will use classic electrophysiology techniques, as well as microelectrode arrays to evaluate the functional consequence of altering cypin levels on neuronal circuit dynamics. Our preliminary results show that neuronal networks that overexpress cypin have less density of functional AMPARs. Understanding the mechanisms that control targeting of PSD-95 at the synapse will shed light into its interactions with other molecular components that influence excitatory and inhibitory synapses and how alterations in this synaptic balance may contribute to cognitive disorders.

Elizabeth Stucky

My overall objective is to use alginate-encapsulated mesenchymal stem cells (MSCs) for prolonged treatment of traumatic brain injury (TBI) and/or ischemic stroke, to ameliorate secondary injury and promote regeneration. Previously, I have developed and optimized an organotypic hippocampal slice culture (OHSC) model of oxygen-glucose deprivation (OGD)
injury to approximate ischemic brain injury. Using this model, I have demonstrated the ability of encapsulated MSCs to prevent degradation of OGD-injured slice cultures. Additionally, I have also used OHSC as a platform to evaluate the ability of encapsulated MSCs to ameliorate the inflammatory response secondary to TBI/ischemia. Using LPS as an inflammatory stimulus, I have shown that encapsulated MSC treatment of LPS-stimulated slice cultures reduces the level of pro-inflammatory cytokine (TNF-α) production, more effectively than treatment with a monolayer hMSC preparation. Recently, I have evaluated the astrocyte- and microglial-specific responses to LPS stimulation, and the ability of encapsulated MSCs to modulate this response. Treatment of astrocyte and microglial cultures with encapsulated MSCs reduced the level of the pro-inflammatory cytokine TNF-α following LPS stimulation, as well as reduced markers of astrocyte reactivity following OGD injury. Ongoing work includes evaluation of the ability of encapsulated MSCs to modulate additional inflammatory mediators produced by these cell populations, as well as to identify the soluble mediators produced by MSCs that are responsible for these changes.

Philip Tedeschi

Cancer cells must satisfy three basic needs for proliferation: ATP for a source of energy, nutrients for macromolecular synthesis, and NADPH for the synthesis of nucleic acids and lipids and the maintenance of redox status in cells. To meet the enhanced need for these macromolecules, cancer cells have an altered metabolism, e.g., aerobic glycolysis rather than oxidative phosphorylation (the Warburg effect), thereby generating high levels of reactive oxygen species (ROS) as compared to normal cells. In order to survive the increase in ROS, cancer cells control oxidative damage primarily through the activities of glutathione reductase and thioredoxin reductase, both of which generate NADPH to function as a reducing agent. Therefore, down regulation of NADPH production in tumor cells is predicted to have a selective and two-pronged negative effect on tumor survival: inhibition of critical biosynthetic pathways and reduction in the ability of cancer cells to handle reactive oxygen species. Our laboratory has recently identified and validated a novel approach to the down regulation of NADPH through the inhibition of NAD kinase (NADK), the only enzyme responsible for generating cytosolic NADP, which is rapidly converted to NADPH mainly by glucose-6-phosphate dehydrogenase (G6PD). We have shown that treatment of cells with NADPS or its prodrug, thionicotinamide, lowered NADPH levels and inhibited cell growth, due in large part to the inhibition of NADK. As a result of the NADPS-induced decrease in NADPH levels, proliferating tumor cells already stressed by high levels of ROS are unable to sustain a further increase in ROS sensitizing them to chemotherapeutic drugs that induce oxidative stress. The importance of NADK in generating NADPH, has been recently recognized as a target for cancer drug development. The objective of our work is to explore the efficacy NADK as a target in cancer, as well as develop potent and selective inhibitors. Our hypothesis is that NADK is a viable target for antitumor drug development. Non-cancerous tissues should be less sensitive to low levels of NADPH, as most are not actively dividing, generate less ROS, and require less robust anabolic pathways. The rationale for this study is the known metabolic differences between cancer and normal cells. Depletion of the NADPH pool by NAK inhibition should allow potent and selective cell kill. We plan to test our hypothesis through an examination of NADK inhibition efficacy in vivo among a panel of murine xenografts in combination with chemotherapy and to rationally develop a new generation of NADK inhibitors using medicinal chemistry and molecular modeling.

Gabriel Yarmush

Given the continuing shortage of transplantable livers, investigators and public health advocates are struggling to develop approached and methodologies to increase the donor pool. One method to accomplish this is to recondition extended criteria donor grafts, a large portion of which are moderate to severe macrosteatotic livers. Transplantation of these livers often leads to primary nonfunction caused by an increased susceptibility to the effects of ischemia reperfusion injury (I/R) that result from the harvesting, transportation, and transplantation of the liver. Our lab has developed a novel procedure to recondition these livers through an ex vivo perfusion protocol that will reduce the hepatic triglyceride content before the onset of the effects of I/R injury. To be implemented in a clinical setting, the defatting process must be completed in a matter of a few hours. While attempts to identify the ideal defatting cocktail in static culture only resulted in defatting livers after days of treatment, the rate of defatting increases to more a clinically relevant timeframe in a flow environment.
My project focuses on understanding the differences in defatting characteristics between a static and flow environment, and using this information to develop the ideal parameters for defatting. We have developed a microchannel perfusion bioreactor to enhance defatting kinetics and investigate defatting under flow. The bioreactors were designed in AutoCAD to hold standard microscope slides that have cells cultured on the slide surface. To date, we have perfused cells with a defatting cocktail for a clinically relevant amount of time (2-3 hours) showing significant defatting. In parallel, we have developed a computational fluid dynamics model of the in vitro flow reactor which will be used to optimize key transport and reaction parameters. The model uses Fluent to determine fluid dynamics and agent uptake into the cells. The results are then ported to a Matlab program in which intracellular fat is tracked along various pathways including triglyceride breakdown, mitochondrial beta oxidation, VLDL secretion, TCA cycle function, and ketogenesis. Once optimal defatting operating conditions are established, we will then determine the effect that defatting under flow has on the viability and function of primary hepatocytes post hypoxia/re-oxygenation (to simulate transplantation). Finally, we are also collaborating with Dr. Guerrera of Columbia University who will use our solutions and operating parameters to perform human liver defatting perfusions.

Agnes Yeboah

A number of skin substitutes have been developed to promote wound healing in acute wounds and in chronic and slow healing wounds. The vast majority of these products consist of some form of barrier on top of a layer or sponge of extracellular matrix (ECM). To improve the performance of these products, growth factors have been added to the treatment scheme. However, these growth factors are very short-lived due to the high levels of proteases found in the harsh wound environment, and normal clearance mechanisms. My research proposes the use of nanoparticle technologies that have improved retention properties and can release these growth factors to help improve wound healing. These nanoparticles consist of a fusion of the peptide growth factors with elastin-like peptides (ELPs). The peptide growth factor-ELP moiety spontaneously self-assembles at physiological temperatures. These nanoparticles offer several advantages: 1) the self-assembly is temperature-reversible, thus enabling rapid and inexpensive purification of the fusion protein; 2) they exclude proteases, protecting the attached biopeptides from degradation; 3) at less than 1 μm, they are small enough to be easily incorporated into existing skin substitutes, which typically have pore sizes in excess of 50 μm; 4) ELPs can be, in principle, attached to any bioactive peptide, thus enabling the generation of different types of nanoparticles that target different components and/or processes of the wound healing process. My hypothesis is that these nanoparticles, appropriately generated and characterized, can significantly enhance the performance of existing skin substitutes. Three specific aims will guide the work: 1) to develop nanoparticles based on bioactive peptide-ELP fusion proteins that target various aspects of the wound healing cascade; 2) to incorporate ELP-based nanoparticles into dermal skin substitutes; 3) to investigate the effect of biological fluids on peptide release from nanoparticles and the immunogenicity of the nanoparticles. The proposed studies will lead to new composite skin substitutes that incorporate nanoparticle-based delivery systems for various growth factors and bioactive peptides. Since the nanoparticles can be incorporated into existing FDA-approved skin substitutes that have already proven beneficial in clinical wound applications, I expect this research to yield new and more effective tissue engineered skin substitutes.

Perry Yin

Recent studies have demonstrated that hyperthermia can be used as an adjuvant that sensitizes tumors to chemotherapy, and can itself induce apoptosis. In particular, one of the most effective methods to achieve a localized hyperthermal effect is to deliver magnetic nanoparticles (MNPs) to the tumor followed by the application of an alternating magnetic field (AMF). However, hyperthermia also results in the expression of heat shock proteins, which in turn, activate survival pathways thereby severely limiting its therapeutic potential. To this end, microRNAs (miRNAs), which are endogenous noncoding RNA molecules that down-regulate or inhibit multiple, and possibly hundreds, of targets, show great promise. In particular, we are interested in let-7, a known tumor suppressor miRNA that targets many pathways up-regulated by hyperthermia including those involved with survival (e.g. PI3K), stress (e.g. p53), proliferation (e.g. RAS), and DNA repair (e.g. BRCA). As such, we hypothesis that targeting these pathways using let-7 will enhance the therapeutic benefits of subsequent
magnetic hyperthermia. Towards this end, we are interested in developing novel magnetic core-shell nanoparticles (MCNPs) for the delivery of let-7 followed by magnetic hyperthermia to enhance the treatment of brain tumor cells. Our MCNPs have a highly magnetic doped MNP core (ZnFe₂O₄) with a noble metal shell (Au). To facilitate let-7 miRNA delivery, the MCNPs were coated with 10 kDa branched polyethyleneimine (PEI) via electrostatic interaction. Finally, to induce magnetic hyperthermia of the MCNPs, we utilized an AMF generator with a fixed frequency of 366 kHz and amplitude of 5 kA/m. Thus far, we have found that the MCNP-based delivery of let-7 results in a significant decrease in cell viability (~60%, p < 0.01) when compared to scrambled miRNA. More importantly, the delivery of let-7 followed by magnetic hyperthermia (45°C for 30 minutes) 24 hours later was found to further decrease the cell viability when compared to magnetic hyperthermia alone (30%, p < 0.05). We are currently investigating the mechanism for this enhanced cell killing effect, and we hypothesize that while IGF1R and PI3K was significantly up-regulated due to hyperthermia, let-7 in the combination therapy is able to suppress these effects. Moreover, we are currently working to optimize our nanoparticle complexes for in vivo studies by coating them with PEG.
PAPERS PUBLISHED


**Lee S, Dong DX, Jindal R, Mitra B, Maguire T, Schloss R, Yarmush ML.** Predicting full thickness skin sensitization using a support vector machine. *Toxicology In Vitro* (Submitted)


Chen M, Przyborowski M, Berthiaume F. Stem cells for skin tissue engineering and wound healing. *Critical Reviews in Bioengineering* 2009; 37:399-421. PMCID: PMC3223487


Dolfi SC, Li-Ying Chan L, Qiu J, Tedeschi PM, Bertino JR. et al. The metabolic demands of cancer cells are coupled to their size and protein synthesis rates: implications for cancer therapies targeting metabolism. *Cancer & Metabolism* 2013; in press


PRESENTATIONS


Faulknor RA, Olekson M, Nativ N, Berthiaume F. Mesenchymal Stem Cells Reverse Hypoxia-mediated Suppression of alpha-Smooth Muscle Actin Expression in Human Dermal Fibroblasts, Rutgers University and Center for Dermal Research, East Brunswick, NJ November 19, 2013 (Poster)

Faulknor RA, Olekson M, Nativ N, Berthiaume F. Mesenchymal Stem Cells Reverse Hypoxia-mediated Suppression of alpha-Smooth Muscle Actin Expression in Human Dermal Fibroblasts, Center for Dermal Research 5th Annual Skin Workshop, Piscataway, NJ November 6, 2013 (Poster)


Fitzgerald KM; Firestein BL. Experimental and modeling approaches to examining neuronal circuitry. Joint Molecular Biosciences Graduate Student Symposium, Piscataway, NJ, Mar 8, 2013.


Jacobs JZ, Zaratiegui M. Sap1 guides the integration of LTR retrotransposons in Schizosaccharomyces pombe, Regional Meeting on Transposable Elements, Cold Spring Harbor, NY, October 26, 2013.


Tedesch PM, Farooqi I, Kathari, YK, Bertino JR. Leucovorin rescue of high dose pralatrexate in mesothelioma xenografts. AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics, Boston, MA. October 20, 2013.


PATENTS


AWARDS

Kate Fitzgerald: New Jersey Commission on Brain Injury Research Graduate Fellowship 2013-2016

Mehdi Ghodbane: National Science Foundation Stem Cell IGERT 2013-2014


Jake Jacobs: Anne B. and James B. Leatham Fellowship 2013-2014

Elizabeth Markensohn (Stucky): NSF IGERT Fellowship 2012-2013

Ileana Marrero-Berrios: Celgene Catherine Program Fellowship 2013-2014

Ilija Melentijevic: Rutgers Excellence Fellowship 2013-2014

Oleg Milberg: NIH Graduate Partnerships Program Fellowship 2012-2014

Nir Nativ: Louis A. Bevier Graduate Fellowship 2013-14

Antoinette Nelson: NSF Alliance for Graduate Education and Professoriate Award 2013

Jillian Nguyen: National Science Foundation Graduate Research Fellowship 2013-15

Jillian Nguyen: Rutgers Dean’s Conference Travel Award 2013

Jillian Nguyen: Rutgers Excellence in Mentorship Fellows Program 2012-2013

Melissa Przyborowski: Women in STEM Research Award, Rutgers Sci Women 2013

Melissa Przyborowski: Conference Travel Award, Rutgers Graduate School Fall 2013

Ana Rodriguez: NSF Innovation through Institutional Integration (I3) Grant Award 2013

Ana Rodriguez: NSF AGEP Travel Grant: Institute on Teaching and Mentoring, Tampa, FL 2012
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<td>Characterization of in-process cell counting in stirred tank bioreactors (STBRs) using the NucleoCounter</td>
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<td>Integra</td>
<td>Evaluating the Effect of Compression Load on Strength of Collagen Sheets</td>
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STUDENT INTERNSHIP REPORT SUMMER 2013

Student Name: Andrea Gray

Department and Advisor: Biomedical Engineering, Dr. Martin Yarmush

Corporation: Celgene Cellular Therapeutics

Mentor: Biren Mistry

Project Title:
Characterization of in-process cell counting in stirred tank bioreactors (STBRs) using the NucleoCounter

General Objective of the Project:
The goal of this work was to characterize the currently used cell counting assay used to track placenta-derived adherent cell (PDAC) growth on suspended microcarriers during the course of large scale culture in STBRs. A parallel objective was to develop a method to determine the quantity of microcarriers in samples taken from STBRs.

Student’s Contribution to the Project:
My work this summer focused on the investigating the effect of sampling parameters on the outcome of the NucleoCounter assay. Additionally I developed an approach to determine the amount of microcarriers in a sample from the STBR using the samples wet weight. When I had time available, I also worked with research to characterize cell health and recovery after cryopreservation.

Techniques Learned:
- Large scale cell culture (10-tray cell factories, STBRs)
- Automated cell counting techniques (ViCell, NucleoCounter)
- Cell health assays (ATP, LDH, Alamar Blue)

Student Comments on the Company and Mentor:
Working at CCT was a great experience. Everyone is friendly, professional and is passionate about doing good science to develop a quality cellular therapy that will alleviate suffering for many patients. My mentor, Biren, was very helpful but still gave me the freedom design the experiments and steer much of the project.
STUDENT INTERNSHIP REPORT SUMMER 2013

Student Name: Paulina Krzyszczyk

Department and Advisor: Biomedical Engineering--Dr. Olabisi

Corporation: Integra LifeSciences

Mentor: Dan Hubbard

Project Title:
Evaluating the Effect of Compression Load on Strength of Collagen Sheets

General Objective of the Project:
The goal of the larger project was to see how varying different parameters of the manufacturing process can lead to stronger sheets for an improved next generation product. Compression load was one of these parameters.

Student’s Contribution to the Project:
Led this particular study from start to end. I developed the idea for this project with my mentor, designed the experimental groups and made the collagen sheets with the team. I submitted the samples for testing, analyzed the results and presented them to the group.

In addition to this project, I contributed to all of the projects going on in the Neuro Product Development lab. This included a project focused on increasing yield of a product in manufacturing and another project involving the design of a wash system that optimizes time and waste volume.

Techniques Learned:
Manufacturing process of collagen products
Product Development
Data Analysis
Design considering "Scaling Up"
Mechanical Testing using Instron

Student Comments on the Company and Mentor:
Dan was a very helpful mentor. He made time to meet with me throughout my internship to check up on my progress and was very accessible. He was open to suggestions and included me as a full member of the team. He made sure that I was exposed to various aspects of the job, including conferences and meetings that brought together different departments at Integra. The entire Neuro Product Development team as a whole was great to work with. I learned a lot from them through our team work.
STUDENT INTERNSHIP REPORT SUMMER 2013

Student Name: Sarah Misenko

Department and Advisor: Biochemistry, Dr. Sam Bunting

Corporation: Celgene Cellular Therapeutics

Mentor: Xiaokui Zhang

Project Title:
Investigating the pharmacokinetics and pharmacodynamics of PDA001

General Objective of the Project:
Generate a reporter system to track PDA001 in vivo during animal studies and optimize PCR detection of low levels of PDA001 in animal tissues.

Student’s Contribution to the Project:

• Evaluate PDA001 cells post-transduction of an HSV-1 reporter system using viability measurements, FACS analysis, and real-time PCR.
• Optimize transfection conditions of suicide gene constructs in PDAC cells.
• Disrupt animal tissues, isolate genomic DNA, and measure detection using digital PCR for optimization of PCR detection.

Techniques Learned:

• Digital PCR
• FACS
• Transfection with Lipofectamine

Student Comments on the Company and Mentor:
The internship program at CCT is very well organized and offered great opportunities to experience various aspects of the company. Dr. Zhang is a very welcoming, helpful, and knowledgeable mentor. This summer at CCT provided great insight into industrial research.
STUDENT INTERNSHIP REPORT SUMMER 2013

Student Name: Antoinette Nelson

Department and Advisor: Biomedical Engineering – Patrick J. Sinko

Corporation: Celgene Cellular Therapeutics

Mentor: Kristen Labazzo

Project Title:
Lung Cell Interactions Project

General Objective of the Project:
The goal of the overall project was to investigate the interactions between placenta-derived adherent cells (PDACs) and the pulmonary environment. Specifically, we studied the ability of these cells to treat pulmonary diseases including idiopathic pulmonary fibrosis and sarcoidosis.

Student’s Contribution to the Project:
I worked independently to develop and implement new assays to study the role of PDACs in facilitating wound healing, cell proliferation and cell migration.

Techniques Learned:

Primary:
- Scratch Assay
- Image J Analysis
- Invasion Migration Assay
- Collagen Drop Assay
- Cel-Titer Glo Cell Proliferation Assay
- Cell Viability Tests
- Enhanced Cell Culturing Skills
- Fluorescent Staining/ Imaging
- Protocol Writing

Secondary:
- Sandwich ELISA
- Flow Cytometry
- cDNA/RNA/DNA extraction for PCR
- Histology

Student Comments on the Company and Mentor:
My time at Celgene Cellular Therapeutics was very enjoyable and lucrative. My mentor allowed me to work independently to strengthen my experimental design skills and critical thinking while always being available for any support needed. The company also required us to conduct presentations and lead journal club seminars for the senior management and principal scientists within the department. We were allowed to not only learn from the scientists around us, but also encouraged to provide our input and initiate thoughtful scientific discussions within the company. It was a wonderful experience.
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# APPENDIX E: BIOTECHNOLOGY TRAINING PROGRAM REQUIRED COURSES

## Topics in Advanced Biotechnology I

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Hints for Success in Graduate School

Ann Stock
CAFM
Biochemistry and Molecular Biology Department
Robert Wood Johnson Medical School
September 20, 2013

What Makes a Great Student in the Lab?

Interviews with PIs:

Charles Craik
Professor
Pharmaceut Chem, Pharmacol, Biochem & Biophys
UCSF

Michael Marletta
Distinguished Professor
Chemistry and Biochemistry
UC Berkeley

Lawrence Marnett
Professor
Cancer Research and Biochemistry
Vanderbilt University

“http://www.benchfly.com/blog/what-makes-a-great-graduate-student/”

September 2010

Qualities of Great Students:

• Professional researchers who take responsibility for their own careers - independence
• Ownership of project
• Passion for answering questions correctly
• Sense of urgency
• Curiosity, inquisitiveness
• Drive, determination, motivation, confidence

Most of these traits can be nurtured, but they cannot be taught.

Develop Essential Skills

• Writing
• Oral presentation
• Critical, analytical thinking

These are fundamental skills for all careers.
Develop Scientific Skills and Knowledge

- Learn a little really well
- Be an expert in something
- Know your own research area in depth
- Know fields outside your own
- Seminars are a great way to gain breadth

Do Not Get Sidetracked by Failures

- Original research is bound to involve failures.
- Appreciate successes, no matter how small.
- Failure should be an expected part of the learning process. However, success requires the ability to learn from one’s mistakes.

The only true cure for failure is success!

Do Not Get Sidetracked by Failures

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- Failure should be an expected part of the learning process. However, success requires the ability to learn from one’s mistakes.

The only true cure for failure is success!

Set Goals and Strive to Reach Them

- Not everything that can be done, should be done
- Focus on high impact research
- Be persistent, but consider when to move on
- Time invested is a sunk cost, not a justification to continue something that is going nowhere
- Keep the big goal in mind
- Develop research approaches that maximize productivity

Overcoming research obstacles is key for success

Reasons that Experiments Fail

- Insufficient technical expertise
- Poor experimental design
- Incorrect hypotheses
- Stupid mistakes!

Identifying the reason for failure is the first step toward correcting the problem.

Acquiring Technical Expertise

- Research requires a diverse set of skills - different for each project.
- Self-assess your expertise and recognize when you lack sufficient knowledge about a technique or method.
- Written protocols are a poor substitute for hands-on guidance.
- Seek help from experts (colleagues in your lab or other labs, special courses).

Develop the skills you need for the job.
Improving Experimental Design

- Include appropriate controls to aid in troubleshooting.
- Plan a logical progression of experiments that establish appropriate parameters (concentration, time, etc.) for the final experiment. Shortcuts rarely save time.
- Predict the results you expect to obtain for each experiment. Simulate data when possible.

Carefully plan experiments to maximize the probability of obtaining useful information.

“Failure is success if we learn from it.”
Malcolm Forbes

“Failure is simply the opportunity to begin again, this time more intelligently.”
Henry Ford

“Failure is simply the opportunity to begin again, this time more intelligently.”
Henry Ford

• Include appropriate controls to aid in troubleshooting.
• Plan a logical progression of experiments that establish appropriate parameters (concentration, time, etc.) for the final experiment. Shortcuts rarely save time.
• Predict the results you expect to obtain for each experiment. Simulate data when possible.

Carefully plan experiments to maximize the probability of obtaining useful information.

Purpose:
Procedure:
Results:
Conclusions:

Put it down in writing. Let your lab notebook be the guide to proper experiment planning and analysis.

“You’ve got to be very careful if you don’t know where you are going, because you might not get there.”
Yogi Berra

“Success is a lousy teacher. It seduces smart people into thinking they can’t lose.”
Bill Gates

“This mouse is running in circles. He has a postdoc phenotype.”

Do not let overcoming a difficult method become the sole goal.
Focus on the scientific aim and consider alternate approaches.

“The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.”
Sir William Bragg

“Dans les champs de l’observation le hasard ne favorise que les esprits préparés.” (Chance favors the prepared mind.)
Louis Pasteur

Know about other fields (courses, seminars, literature, meetings).
Take ownership of your project and don’t be afraid to think outside the box.
"The two most common elements in the universe are hydrogen and stupidity."

**Minimizing Mistakes**

- Don’t mistake hours in the lab for productivity.
- Plan experiments to avoid random, unconsidered or rushed decisions.
- Plan your time so that procedures are not rushed.
- Get some sleep! Take a break!

Put quality time into your experiments.

*Just showing up is not enough.*

**OWN YOUR PROJECT!**
Altering Neuronal Circuitry: Experimental and Modeling Approaches

Kate Fitzgerald
Advisor: Dr. Bonnie Firestein
20 September 2013

Outline

- Introduction
- Motivation
- Background
- Methods
- Results
- Future Work
- Summary

Introduction

DENDRITE BRANCHING

- Interested in overall branching dynamics and in protein-protein interactions that promote/prevent branching

Background

Communication among Neurons

- Why dendrite branching?
  - Communication among neurons will not occur without...
    1) Proper dendrite morphology
    2) Accurate protein trafficking to the synapse
  - Integration of inputs from other neurons determines how that neuron functions

Motivation

Dendrite Branching in Pathology

- Why do we care?
  - Implicated in disease
  - Aberrant changes in morphology cause functional deficits
Examining Morphology Reveals Nothing about Functionality

Figure 5: Changes in morphology and viability after increasing doses of NMDA exposure. [Tseng thesis, 2011]

Examining Morphology Reveals Nothing about Functionality

Figure 6: Changes in synchronization after glutamate treatment. [Kutzing et al., ABME 2011]

Background

Brain-derived Neurotrophic Factor (BDNF)

- One of the most critical factors for dendrite branching and outgrowth
- Development
- Plasticity
  - Participates in both early [Gartner et al. 2006] and late phase [Pang et al. 2004] long-term potentiation
- Pro-survival factor
  - Overexpressed in cells that survive ischemic and hypoxic stress [Beck et al. 1994]

Global BDNF Promotes Proximal Branching

Figure 7: Global BDNF increases proximal branching. [Kwon et al., J Neurosci, 2011]

Local BDNF Promotes Proximal AND Distal Branching

Figure 8: Local BDNF increases proximal and distal branching. [unpublished data]

Background

There exist two different mRNA transcripts for BDNF that encode the same protein

- Why the difference?
- Different length 3’ UTRs cause differential localization of the transcripts
  - Short 3’ UTR is localized to soma
  - Long 3’ UTR is localized to both soma and dendrites
- Physiological significance?
**Biotechnology Training Program**

**Background**

**BDNF and Neuroprotection**

- Glutamate levels spike immediately after injury, remain slightly elevated
  - One hypothesis why NMDAR antagonists failed in clinical trials is that they block the excitatory synaptic transmission that was actually necessary for survival
- Synaptic and extrasynaptic NMDARs have different functions
  - Extrasynaptic NMDARs shut off CREB pathways and prevent BDNF signaling
  - Synaptic NMDARs do not

*Ikonomidou & Turski, Lancet Neurol 2002*  
*Hardingham et al., Nature 2002*

**Aims**

**Broad Objectives**

1) Determine how BDNF affects network dynamics.
   - Different exogenous distributions of BDNF (global vs. local application)
   - Unique endogenous sources of BDNF (long vs. short transcript)
   - Hypothesis: Since BDNF promotes branching, we expect it will cause an increase in synchronization among electrodes without altering overall activity.

2) Assess whether BDNF is able to protect and/or rescue functional deficits caused by glutamate-induced excitotoxicity.
   - Hypothesis: As BDNF is known to be a pro-survival factor, we expect 1) its overexpression prior to injury to prevent synchronization decrease and 2) its application after injury to rescue some synchronization.

3) Model the above scenarios using the Fitzhugh-Nagumo model of neuronal activity to determine what network properties result in the observed phenomena.

**Methods**

**Dissociated Hippocampus from Rat Embryos at the 18th Day of Gestation (E18)**

- At E18, the neurons from the hippocampus that survive in culture are mostly CA3 neurons that have just exited the cell cycle
  - This provides us with a relatively homogeneous population of neurons
  - Physiological functions of learning and memory
  - Allows us to study developing neurons as they mature in culture

**Microelectrode Arrays (MEAs)**

- MEAs are a noninvasive method for measuring neuronal network activity over time
- Neurons are plated at a high density
  - 1 million cells per MEA → 350,000 cells / cm²

**Data Collection**

- Sampling frequency during recording is 20,000 Hz
- Record for 5 min at a time
- Use recording media to regularize bursting
  - 10 mM K⁺

**Data Analysis**

- **Step 1:** detect spikes
- **Step 2:** determine if spikes are part of a burst
- **Step 3A:** determine if any bursts on different electrodes overlap with one another
- **Step 3B:** use this to calculate a raw correlation
- **Step 4:** Normalize raw correlation by the maximum number of bursts to determine synchronization index

*Kutzing et al., ABME 2011*  
*Wagenaar thesis, 2005*
Results: TrkB Expression

TrkB-FL Receptor Expression in Hippocampal Cultures

* = record & treat
R = record

Results: Global BDNF

Global BDNF – Network Activity

Methods

Data Analysis: Automated

Hodgkin-Huxley Model

\[ I = C_r \frac{dV}{dt} + \beta \delta (V - V_m) + \beta \delta (V - V_n) \]

\[ V = V - V_a / (3 + W + I) \]

FitzHugh-Nagumo Model

\[ \frac{dW}{dt} = V - W^3 / 3 - W + I \]

\[ W = 0.08(V + 0.7 - 0.8W) \]

Collaboration with Dr. Troy Shinbrot
Challenges with the Model

• Nonlinear dynamical system

• In vitro, we record from electrodes, not from neurons
  – Can average signal from neurons to simulate “electrodes”

• Ultimate goal:
  – To be able to model changes seen after treatment or injury in culture by altering particular parameters

Collaboration with Dr. Troy Shinbrot

Summary & Future Directions

Summary

• Investigation of how BDNF alters network activity is motivated by our knowledge of how BDNF alters the dendritic arbor

Future Directions

• Determine how overexpression of BDNF with short and long 3' UTR affects dendrite branching dynamics (ongoing)
• Develop virus for these constructs so that they can be used for functionality studies
• Clarify behavior of the model and incorporate it with experimental data

Acknowledgements

Advisor: Dr. Bonnie Firestein
Collaborator: Dr. Troy Shinbrot
Current lab members:
  – Ana Rodriguez
  – Kristina Hernandez
  – Kara Mann
  – Chen Liang
  – Mihir Patel

Altering Neuronal Circuitry: Experimental and Modeling Approaches

Kate Fitzgerald
Advisor: Dr. Bonnie Firestein
20 September 2013

Methods

Data Analysis: Activity Detection

1) Spikes are detected according to predetermined threshold
  – I am working on developing an adaptive thresholding method

2) Timing of spikes is compared to determine whether they are part of a “burst”
  – There must be at least 4 spikes closely spaced together
  – Firing rate of those spikes must be at least 4x the average firing rate on that electrode
  • Or they need to be spaced 100 ms apart (whichever is smaller)

Data Analysis: Synchronization Calculation

1) Synchrony of firing (SF) is calculated between two electrodes
  – Based on the number of times the electrodes fire together vs. maximum number of times they fire

\[
SF = \frac{B_{xy}}{B_{CF}}
\]

2) SF is normalized to produce a synchronization index (SI)

\[
SI = \left(1 - \left(1 - \frac{X_{xy}}{M_{MB}N_{NB}}\right)^{MB}MB\right)^{SF}
\]

– SF is weighted by a factor that compares the number of times the two electrodes record overlapping bursts \(N_{xy}\) compared to the number of bursts \(N_{NB}\) in that particular recording period and weighted by the maximum number of bursts \(MB\) recorded on that MEA.
**Methods**

Data Analysis: Synchronization Calculation (cont’d)

1. We can then generate average synchronization (AS1) plots
   - AS1 is assumed over each row (or column) and weighted by the maximum number of active electrode(s).
   - Then if iF = 1 in MEA, otherwise 0 elsewhere, the AS1 would still be 1
   - Location on plot corresponds to actual physical location of electrode on MEA

\[
AS1 = \sum_{i} S_{ij} / N_{E1}
\]

- Future improvements:
  - Add additional parameters:
    - Clustering coefficient
    - Path length
    - Measuring of firing rate and/or inter spike intervals
    - Citations

**Previous Work**

Glutamate Injury Studies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Long Proximal</th>
<th>Proximal BR1</th>
<th>Proximal BR2</th>
<th>Total</th>
<th>MEA Fractionality</th>
</tr>
</thead>
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<td>$\uparrow$</td>
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</tr>
</tbody>
</table>

**Background**

Brain-derived Neurotrophic Factor (BDNF)

- BDNF increases proximal branches
- Overexpression of TrkB-FL increases proximal
- Overexpression of truncation T1 lengthens distal dendrites
Characterization, Fabrication, and Application of 3-D Thermo-reversible Collagen Hydrogels

Kathryn Drzewiecki
Biomedical Engineering
Advisor: Dr. David Shreiber
September 19th, 2013

Biomaterials for Tissue Engineering

Type-I Collagen
- Self assembles
- Can be modified through side-chain chemistry

Collagen Methacrylamide (CMA)
Photocrosslinkable Collagen for the spatiotemporal control of bioactive/mechanical properties
CMA Rheology

Modify the mechanical properties of CMA using UV light.
Gaudet et al, 2012

CMA Applications – Artificial Skin

Use CMA to locally increase strength and stiffness
Figure adapted from Reichmann E., et al. 2005

‘Cold Melt’ of CMA

Type I collagen remains a fibrillar network, while CMA reversibly cold melts!

Objective

1. Is CMA a thermo-reversible hydrogel? How does CMA dis-assembly and re-assembly occur?

2. Can we fabricate 3D gels of CMA, similar to 3D printing, for tissue engineering applications?

Specific Aim 1

Specific Aim 1: Characterize CMA thermo-reversible self-assembly at a molecular and bulk structural level, and determine the mechanism.

- Characterization of mechanical properties doing cold melting
- Characterization of secondary and higher order structure using circular dichroism and dynamic light scattering, respectively
- Molecular modeling of collagen and CMA sequences

What structural differences explain CMA’s thermo-reversibility?

Secondary Structure Characterization

Melt curve at 222 nm
4 °C
Both collagen and CMA are triple helical.
Self-assembled – 37 °C

37 °C Collagen and CMA lose triple helical structure.

Cold denatured – 4 °C

4 °C CMA regains triple helicity and collagen does not.

Re-assembled – 37 °C

37 °C CMA loses triple helicity.

Fully denatured – 50 °C

50 °C Collagen and CMA lose all secondary structure.

DLS ‘Cold Melt’

CMA assembly is delayed compared to collagen.

DLS ‘Cold Melt’

CMA size begins to decrease as it cold melts, while collagen size distribution stays stable.
CMA size begins to increase as it re-assembles, and has a larger size distribution than at initial assembly.

CMA appears more rigid than collagen!

Stronger hydrogen bond stabilizes CMA.

Summary of Specific Aim 1

- Characteristics of CMA are rapidly **thermo-reversible** at multiple scales:
  - Secondary structure (CD) – triple helix
  - Tertiary structure (DLS) – self-assembly into larger species
  - Mechanical properties (rheology) – stable hydrogel

- The **loss of triple helix** in collagen and CMA appears to lead to self-assembly in DLS and rheology.

- Molecular modeling shows that the stability of CMA may be a result of stronger hydrogen bonding.

Complete CMA Characterization

- Compare properties of hydrogels following re-assembly (fibril size, mechanical/bioactive crosslinking abilities)
- Stacking of aromatic residues
**Future Work - Specific Aim 1**

- Determine thermo-reversibility mechanism
  - Differences in self-assembly
    - Stacking of aromatic residues
    - Osmotic differences

---

**Objective**

Can we fabricate 3D gels of CMA, similar to 3D printing, for tissue engineering applications?

---

**3D Printing**

Limitations

- Most use synthetic polymers
- Expensive
- No printing at nanometer scale

---

**How is CMA thermo-reversible?**

Potential differences in "hydration shells" around individual CMA triple helices may cause potential structural differences in self-assembly, and then water interactions as the material cools.

Bella et al, 1995
**Specific Aim 2**

3D printer + collagen = body part

"Bioengineers print ears that look and act like the real thing." Cornell University, 2/20/13

**Specific Aim 2**

- Additive printing
  - Creation of any 3D shape
  - Successive layers of materials added
- Subtractive printing
  - Create shapes by removing material
  - 2D or 3D UV light

**Specific Aim 2**

- How much CMA is needed for cold melting?
- How much photoinitiator is necessary for crosslinking?
- What spatial resolution do we have?
- What scale are we working on?
  - Organs vs. microniche

**Specific Aim 3**

Specific Aim 3: Understand general cell interactions with CMA and drive specific cell behavior with fabricated scaffolds.

How do cells behave in a thermo-reversible hydrogel?
- Can gels still dis-assemble?
- What happens to the gel?
Specific Aim 3: Understand general cell interactions with CMA and drive specific cell behavior with fabricated scaffolds.

Can we control specific cell behavior?

- Guarantee viability – optimize crosslinking
  - Proliferation
  - Migration into scaffolds
  - Differentiation
  - Gene expression
  - Focus on a particular organ design

Future Work

- Complete CMA characterization
  - Comparisons of CMA before and after re-assembly through SEM and crosslinking abilities
- Determine differences between fibril associations in collagen and CMA
  - Aromatic stacking
  - Osmotic differences
- Begin simple free form fabrication of CMA gels in 2D

Acknowledgements

- Shreiber Lab
- Nanda Lab
- Rutgers Biotechnology Training Program
- IGERT on Stem Cells

Questions?

References


Why is CMA thermo-reversible?

Is it the methacrylic acid?

- Coupling methacrylates at different concentrations or working with different concentrations of collagen
  - Vary dis-assembly and re-assembly temperatures
- Molecular modeling of other organic compounds coupled to collagen
- Design a library of biomaterials with similar properties to CMA
Potential Research Aim 2

Benefits
- Thermo-reversible
- Control of Chemical/Mechanical properties
- Self-assembly

Limitations
- No dis-assembly if crosslinked
- Radical Generation

Potential Research Aim 3

Can we use the thermo-reversible self-assembly of CMA to study cell-matrix interactions?

- Study cell cytoskeletal rearrangement following dis-assembly
- What happens to material when gel dis-assembles?
- Can we study other fibril-forming collagens?

Potential Research Aim 4

Free Form Fabrication of Methacrylated collagen

3D cyto-architectures

Layer-by-layer deposition

What method can we use to create complex architectures for tissue transplants?

CMA Potential as a new biomaterial

- Schwann Cells
- Mesenchymal Stem Cells
- Encapsulate cells in CMA gels
- Preserve through freezing

Duan et al, 2010
Bruning et al, 2009
Synthesis of Collagen Methacrylamide

Couple MA carboxyl with free amines on side chains of lysine residues, N-termini

Result: Collagen Methacrylamide (CMA)

CD 'Melt' – Self-assembled – 37 °C

Wavelength scan at 37 °C

Collagen and CMA lost triple helicity.

CD 'Melt' – Cold Denatured – 4 °C

Wavelength scan at 4 °C

CMA re-gained triple helicity!

CD 'Melt' – Heat Denatured – 50 °C

Wavelength scan at 50 °C

CMA loses triple helicity again, while collagen secondary structure is stable.

CD 'Melt' – Re-assembled – 37 °C

Wavelength scan at 37 °C

CMA loses triple helicity again, while collagen secondary structure is stable.
3Ps: Proposals, Presentations, and Papers

Martin Yarmush, MD, PhD
Rutgers Biotechnology Training Program
125:16:603 "Topics in Advanced Biotechnology I"
October 4, 2013

Taking Responsibility For Your Project

• It is crucial that you understand this as soon as possible!!
• Most important transition from your undergrad years
  (true learning and development happens in your PhD)
• By taking responsibility for your project you will develop
  strong skills in independent and thinking, critical analysis,
  problem-solving, and time management.
• Own your project: this means it’s yours to grow and serve
  (be open to feedback, comments, criticism)
• Tenacity and persistence are essential! Keep at it! Never
  give up!

Proposals: Focus on your Audience

• Write a brief letter to a friend asking him/her to send you
  $1000.
• Why do you need the money?
• How important is it?
• How far along are you toward your goal?
• What will you do with the money?
• What will he/she get out of it?

What will your readers want?

• Substantive content
  – clear and compelling purpose
  – knowledge of the field
• Organization that makes key information easy to find
• A professional finish (good grammar, correct punctuation,
  neat appearance, correct citations)

Thesis Proposal

– Perhaps the most crucial stage; everything flows
  from here. Later problems can often be traced
  back to a weak thesis proposal.
– This is where you need your advisor and others
  the most (FREE CONSULTING!)
– Main challenge: come up with a problem
  statement (usually in the form of an hypothesis),
  and an approach to solve your problem
– It’s your GPS and will make the trip faster and
  easier

NIH Proposal Component Parts

• Background and Significance:
  – Why do I care, why is this important
  – If important, what has been done (current knowledge or state of
    the field
  – Gap in the knowledge base or unmet need
• Innovation:
  – A new and substantially different way of addressing an
    important problem which enables departure from the status quo
  – For example: Does the project challenge existing paradigms or
    clinical practice; address an innovative hypothesis or critical
    barrier to progress in the field? Does the project develop or
    employ novel concepts, approaches, methodologies, tools, or
    technologies for this area?
• Approach:
  – Give objective and any preliminary results; how will you conduct
    your studies and why this approach (rationale); what do you
    expect and what if things don’t work
The Hypothesis
- Gene xyz was identified in a microarray experiment comparing normal breast epithelial cells to breast cancer
- Gene xyz is a rare isozyme of pyruvate kinase expressed in embryonic stem cells
- xyz expression allows breast cells to grow on soft agar

Objective: To determine if xyz plays a causal role in breast cancer, and how it works (broad)

Hypothesis: Xyz regulates glycolytic pathways that confer properties of self-renewal to breast epithelial cells (specific and mechanistic)

The Aims
- Collectively they test the hypothesis
- Independent, complementary approaches
- Can be interdependent but not dependent
- Are related – flow together logically
- Can be accomplished with time/$ requested

Bioengineering: IRE and Skin
- We hypothesize that high frequency pulsed electric field treatment (non-thermal) of skin will trigger scarless regeneration of the treated area by preserving the ECM and the local vasculature.

Anatomy of the Specific Aims
- Introductory paragraph
  - General to specific
  - Why do I care, why is this important
  - If important, what has been done (current knowledge or state of the field
  - Gap in the knowledge base or unmet need
- Individual aims
  - 2-4 (Give objective, rationale, how you will conduct your studies
- Closing paragraph
  - Can be useful to emphasize the potential impact of this grant to the field

Example Aims

Hypothesis: Xyz regulates glycolytic pathways that confer properties of self-renewal to breast epithelial cells
- To determine if xyz differentially regulates the glycolytic, hexosamine, and pentose phosphate pathways in breast epithelial cells
- To determine if xyz expression confers properties of self-renewal and tumorigenicity to breast epithelial cells
Summarize the approach

- Give the reader a sense of how you will address the aim
- Catch their attention by highlighting novel or innovative approaches
- Give enough specifics to jog their memory after they read the rest of the grant

Example

Hypothesis: Xyz regulates glycolytic pathways that confer properties of self-renewal to breast epithelial cells

Aim 1
- To determine if Xyz differentially regulates the glycolytic, hemosamine, and pentose phosphate pathways in breast epithelial cells. Metabolic flux analysis with radiolabeled carbohydrate precursors and MS-MS will be used to analyze primary normal breast epithelial cells. Cells will be modified with Lentivirus constructs of Xyz and PK in order to identify and quantitate alterations in the rate of production and composition of cellular metabolites.

Dependent Aims

- This means that subsequent aims are dependent on the outcome of the work proposed under the first aim
  - if aim #1 is not successful then the whole project falls apart
  - this is viewed as a “fatal flaw” in a research proposal
  - ALL aims must be achievable

Example of Dependent Aims

- Aim 1: To determine if drug A interacts with drug B
- Aim 2: To determine the mechanism for the interaction between drug A and drug B

Your ability to achieve aim 2 is completely dependent on the success of aim 1
These are also broad, non-descript aims that may not be achievable

Anatomy of the Specific Aims

- Introductory paragraph
  - General to specific
  - Why do I care, why is this important
  - If important, what has been done (current knowledge or state of the field
  - Gap in the knowledge base or unmet need
- Individual aims
  - 2-4 (Give objective, rationale, how you will conduct your studies
- Closing paragraph
  - Can be useful to emphasize the potential impact of this grant to the field

Effective Presentations

- Basic rule
  - Say what you are going to say
    - 1-3 main points in the introduction
  - Say it
    - Give the talk
  - Then say what you said
    - Summarize main points in the conclusion
### Audience
- Why and to whom are you giving this presentation?
- What do you want the audience to learn?

### Tell a Story
- Prepare your material so that it tells a story logically
  - Introduction/overview
  - Method/approach
  - Results/analysis
  - Conclusion/summary
- Create continuity so that your slides flow smoothly
  - Guide the audience through your story
  - Your last point on one slide can anticipate the next slide

### Methods
- Methods, Instrumentation
  - For most talks, only present the minimum
  - Broad overview of how things work

### Results
- Data Tables
  - Tables are useful for a small amount of data
- Figures
  - ‘1 figure = 1000 words’
  - Figures should be readable and uncluttered
  - Keep figures simple, use color logically for clarification
  - Explain axes, variables, statistics
- Summary Cartoon
  - Major findings or an illustration of the processes or problem (Consider showing it at the beginning and the end)

### Preparing the Presentation
- Average not more than 1 slide per minute
- Logical animations are OK
- Use 3-7 bullet points per page
  - Avoid writing out, and especially reading, long and complete sentences on slides because it is really boring to the audience
- Slide appearance (font, colors) should be consistent
- Spellcheck!!

### Font Size
Type size should be 18 points or larger:
- 18 point
- 20 point
- 24 point
- 28 point
- 36 point

AVOID USING ALL CAPITAL LETTERS BECAUSE IT’S MUCH HARDER TO READ

*References can be in 12-14 point font*
**Tips**

- Practice – actually stand up and say the words out loud
- Starting out is the hardest part of the talk (ending is the second hardest) – prepare both
- Stand where the figures can be seen
- Look at people during presentation
- Be enthusiastic
- Don’t rush
- Don’t forget acknowledgements, always give proper credit
- At the end come back to the big picture and summarize the significance of your work in that context; open up new perspectives

**Questions**

- Questions help you
  - Identifies parts the audience did not understand
  - May refocuses or adds new dimensions
- You can repeat the question
  - This gives you time to think
  - The rest of the audience may not have heard the question
  - Also if you heard the question incorrectly, it’s an opportunity for clarification
- Keep your answers short and to the point – don’t respond with another lecture
- Anticipate typical questions and prepare for them
  - Make extra slides – perhaps on details of instrumentation or methodology
- If you really don’t know the answer
  - Say “Interesting, I will look into that” or “That’s a good point, let’s discuss it afterwards”
- If the questioner disagrees with you and it looks like there will be an argument then diffuse the situation
  - “We clearly don’t agree on this point, let’s go on to other questions and you and I can talk about this later”
Previous work identified glycine as a important metabolite in cancer cell proliferation.

Mitochondrial folate enzymes were also linked with proliferation.

Protein synthesis requirements for serine and glycine do not match uptake measurements.

Too much serine, too little glycine.

Combining the two matches with expected demand.

'SOG' pathway.
Conclusions

• SOG pathway expression is correlated with proliferation
  • Pathway primarily supports ATP, NADPH and purine synthesis

• New MoA for methotrexate (short time scale)

• Proposed novel findings
  • Hypoxia induces uptake and synthesis (via Warburg) supports purine synthesis, and ATP and NADPH generation
  • MTHFD1 and MTHFD1L
  • MTHFD1 is an important source of ATP/NADPH in cytosol
Nanoscale Approaches for HIV Eradication and Prevention

Antoinette Nelson
Advisor: Dr. Patrick J. Sinko
Fall 2013

New HIV infections = ~ 2.7 million [2.4 – 3.0 million]
Deaths due to AIDS = ~ 2.0 million [1.7 – 2.4 million]

HIV Virus

HIV-1 Susceptible Cells
- T-cells
- Monocytes
- Macrophages
- Dendritic Cells
- Microglial cells

Types of Anti-HIV Therapeutics
- Nucleoside reverse transcriptase inhibitors
- Non-nucleoside reverse transcriptase inhibitors
- Protease inhibitors
- Fusion inhibitors
- HAART (Highly Active Antiretroviral Therapy)

Obstacles for HIV Therapy
- Macrophages, T-cells and dendritic cells occupy areas difficult to penetrate with drugs
- Occupy the brain, lung, lymph node, spleen, bone marrow, GI tract
- Macrophages more resistant to the cytopathic effects of the virus
- Cellular reservoir for long term persistence of HIV-1
- Produces and harbors the virus for a longer period
- HAART efficiency limitation

98% of lymphocytes are distributed among the lymphoid tissues
- GALT: gut associated lymphoid tissues
- Protect body from invasion
- Digestive system is an important component of immune system
- Lymphoid tissues heavily infected upon initial infection
- Sustained drug release needed
Challenge
To eliminate residual reservoirs of HIV that persist in individuals despite chronic antiretroviral therapy

Project Goal
To develop nanocarrier-based approaches to eliminate active cellular reservoirs of HIV in the gut and mesenteric lymph nodes by delivering antiretroviral agents locally in effective and sustained concentrations to inhibit HIV transmission by blocking viral entry and disrupting viral synapses

Flash Nanoprecipitation

Nanoparticle

NP Formation

PS-PEG Block Copolymer
Hydrophilic Block
Drug
Imaging Agent
Hydrophobic Block

Flash Nanoprecipitation

Organic Stream
Anti-solvent (Water)
Nanoparticles
Mixing Chamber ~100 μl

Distribution of NPs in Foam

Non-Alcoholic Foam at Room Temperature

30 sec
5 min
15 min
30 min
1 h
2 h
Optical and MRI Images of Foam Distribution in Colon

Acknowledgements

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Dr. Patrick J. Sinko
Dan Myers
Mahta Samidezah
Zoltan Szekely, PhD
Steven Johnson
Xiaoping Zhang, PhD
Derek Adler

Project Goals

1. Optimize surface ligands
2. Systemically vary components
3. Perform in vitro binding and transport tests
4. Formulate and assess foam distribution and residue
An Overview of Basic Statistics
Ramsey A. Foty, Ph.D.
Department of Surgery
Rutgers-RWJMS
Biotech Special Topics-BME

First....some perspective

"An unsophisticated forecaster uses statistics as a drunkard uses lamp-posts-”
"Then there is the man who drowned crossing a stream with an average depth of six-inches"
W.E. Gates. . . German Author
"Statistics: The only science that enables different experts using the same figures to draw different conclusions."
Evan Esar. . . American Humorist
"There are three kinds of lies: lies, damned lies, and statistics."
Mark Twain/Benjamin Disraeli.

Why do we need statistics?

• Variability is the norm.
• Variability can obscure important findings.
• We naturally assume that observed differences are real and not due to natural variability.
• Statistics allow us to draw from the sample, conclusions about the general population.

Topics

• Sample vs population.
• Gaussian/normal distribution.
• Descriptive Statistics.
  • Measures of Location.
  • Mean, Median, Mode.
  • Measures of Dispersion.
  • Range, Variance, Standard Deviation.
  • Precision of the mean.
  • Standard Error, Confidence Interval.
• Outliers.
• Grubb’s test.
• The null hypothesis.
• Comparing two means.
  • T-test (parametric).
• Comparing 3 or more groups.
  • ANOVA.
• Linear Regression.
• Power Analysis.

Sample vs Population

• Taking samples of information can be an efficient way to draw conclusions when the cost of gathering all the data is impractical.
• If you measure the concentration of factor X in the blood of 10 people, does that accurately reflect the concentration of Factor X of the human race in general? How about from 100, 1000, or 10,000 people? How about if you sampled everyone on the planet?

Statistical methods were developed based on a simple model:

• Assume that an infinitely large population of values exists and that your sample was randomly selected from a large subset of that population. Now, use the rules of probability to make inferences about the general population.
Statistics come in two basic flavors

**Parametric**
- A class of statistical procedures that rely on assumptions about the shape of the distribution (i.e., assume a normal distribution) in the underlying population and about the form or parameters (i.e., means and standard deviations) of the assumed distribution.

**Non-parametric**
- A class of statistical procedures that do not rely on assumptions about the shape or form of the probability distribution from which the data were drawn.

**The Gaussian Distribution**

If samples are large enough, the sample distribution will be bell-shaped. The Gaussian function describing this shape is defined as follows:

\[ f(x) = \frac{1}{\sqrt{2\pi \sigma^2}} e^{-\frac{(x-m)^2}{2\sigma^2}} \]

where \( m \) represents the population mean and \( \sigma \) the standard deviation.

**Descriptive Statistics**

**Measures of Location**
- A typical or central value that best describes the data.
  - Mean
  - Median
  - Mode

**Measures of Dispersion**
- Describe spread (variation) of the data around that central value.
  - Range
  - Variance
  - Standard Deviation
  - Standard Error
  - Confidence Interval

No single parameter can fully describe distribution of data in the sample. Most statistics software will provide a comprehensive table describing the distribution.

**Measures of Dispersion**

- Describe the spread or variability within the data.
- Two distinct samples can have the same mean but completely different levels of variability.
- Which mean has a higher level of variability?
  - 110 ± 5 or 110 ± 25
- Typical measures of dispersion include Range, Variance, and Standard Deviation.
Measures of Dispersion: Range

**Range**
- The difference between the largest and smallest sample values.
- It depends only on extreme values and provides no information about how the remaining data is distributed.

For the cell migration data:
- Largest distance = 200 microns
- Smallest distance = 24 microns
- Range = 200 - 24 = 176 microns.

NOT a reliable measure of dispersion of the whole data set.

Measures of Dispersion: Variance

**Variance**
- Defined as the average of the square distance of each value from the mean.
- To calculate variance, it is first necessary to calculate the mean score, then measure the amount that each score deviates from the mean.

The formula for calculating variance is:

$$S^2 = \frac{\sum (X - M)^2}{N-1}$$

**Why Square?**
- Squaring makes them all positive numbers (to eliminate negatives, which will reduce the variance).
- Makes the bigger differences stand out, 100² (10,000) is a lot bigger than 50² (2500).

Measures of Dispersion: Standard Deviation

**Standard Deviation**
- The most common and useful measure of dispersion.
- Tells you how tightly each sample is clustered around the mean. When the samples are tightly bunched together, the Gaussian curve is narrow and the standard deviation is small.
- When the samples are spread apart, the Gaussian curve is flat and the standard deviation is large.

The formula to calculate standard deviation is:

$$SD = \sqrt{\frac{\sum (X - \bar{X})^2}{N-1}}$$

For the cell migration data:
- Size of the population N
- Size of the sample N-1

For the cell migration data, the sample variance is:

$$S^2 = \frac{\sum (X - M)^2}{N-1}$$

NOT a very user-friendly statistic.
For this data set, the mean and standard deviation are:

77 ± 57 microns

Conclusion: There’s lots of scatter in this data set.

But then again....

- This is a fairly small population (n=9).
- What if we were to count the migration of 90, or 900, or 9000 cells.
- Would this give us a better sense of what the average migration distance is?
- In other words, how can we determine whether our mean is precise?

### Precision of the Mean

**Standard Error**

- A measure of how far the sample mean is away from the population mean.

\[ SEM = \frac{SD}{\sqrt{N}} \]

*SEM* gets smaller as sample size increases since the mean of a larger sample is likely to be closer to the population mean.

For our data set:

\[ SEM = \frac{57}{\sqrt{9}} = 19 \]

Increasing sample size does not change scatter in the data. SD may increase or decrease. Increasing sample size will, however, predictably reduce the standard error.

### Should we show standard deviation or standard error?

**Use Standard Deviation**

- If the scatter is caused by biological variability and you want to show that variability.
- For example: You aliquot 10 plates each with a different cell line and measure integrin expression of each.

**Use standard error**

- If the variability is caused by experimental imprecision and you want to show the precision of the calculated mean.
- For example: You aliquot 10 plates of the same cell line and measure integrin expression of each.

### Precision of the Mean

**Confidence Intervals**

- Combines the scatter in any given population with the size of that population.
- Generates an interval in which the probability that the sample mean reflects the population mean is high.

The formula for calculating CI:

\[ CI = \bar{X} \pm (SEM \times Z) \]

- \( \bar{X} \) is the sample mean and \( Z \) is the critical value for the normal distribution.
- For the 95% CI, \( Z=1.96 \).
- For our data set: 95% CI=77 ± (19x1.96)=77 ± 37.2

\[ 95\% CI=77-90 \]

This means that there’s a 95% chance that the CI you calculated contains the population mean.

### CI: A Practical Example

<table>
<thead>
<tr>
<th>Data set A</th>
<th>Data set B</th>
<th>Data set A</th>
<th>Data set B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>86.1</td>
<td>64.1</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>4.1</td>
<td>19.3</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.3</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Low 95% CI</td>
<td>83.2</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>High 95% CI</td>
<td>89.9</td>
<td>77.9</td>
<td></td>
</tr>
</tbody>
</table>

Between these two data sets, which mean do you think best reflects the population mean and why?
SD/SEM/95% CI error bars

Outliers
- An observation that is numerically distant from the rest of the data.
- Can be caused by systematic error, flaw in the theory that generated the data point, or by natural variability.

How to deal with outliers?
- In general, we first quantify the difference between the mean and the outlier, then we divide by the scatter (usually SD).

Grubb's test
\[ Z = \frac{\text{mean} - \text{value}}{\text{SD}} \]

For the cell migration data set:
The mean is 77 microns. The sample furthest from the mean is the 200 micron point and the SD is 57. So:
\[ Z = \frac{77 - 200}{57} = -2.16 \]

What does a Z value of -2.16 mean?
- In order to answer this question, we must compare this number to a probability value (P) to answer the following question:
  - “If all the values were really sampled from a normal population, what is the chance of randomly obtaining an outlier so far from the other values?”
- To do this, we compare the Z value obtained with a table listing the critical value of Z at the 95% probability level.
- If the computed Z is larger than the critical value of Z in the table, then the P value is less than 5% and you can delete the outlier.

For our data set:
- Z calc (2.16) is less than Z Tab (2.21), so P is greater than 5% and the outlier must be retained.
Hypothesis Testing

- Observe Phenomenon
- Propose Hypothesis
- Design Study
- Collect and Analyze Data
- Interpret Results
- Draw Conclusions

Statistics are an important part of the study design.

The Null Hypothesis

- Appears in the form \( H_0: \mu_1 = \mu_2 \)
  Where: \( H_0 \) = null hypothesis
  \( \mu_1 \) = mean of population 1
  \( \mu_2 \) = mean of population 2
- An alternate form is \( H_1: \mu_1 - \mu_2 = 0 \)
- The null hypothesis is presumed true until statistical evidence in the form of a hypothesis test proves otherwise.

 Statistical Significance

- When a statistic is significant, it simply means that the statistic is reliable.
- It does not mean that it is biologically important or interesting.
- When testing the relationship between two parameters we might be sure that the relationship exists, but is it weak or strong?

Strong vs weak relationships

Comparing Two Means

- Are these two means significantly different?

Comparing Two Means

- Variability can strongly influence whether the means are different. Consider these 3 scenarios: Which of these will likely yield significant differences?

Comparing Two Means

Student t-test

- Introduced in 1908 by William Sealy Gosset.
- Gosset was a chemist working for the Guinness Brewery in Dublin.
- He devised the t-test as a way to cheaply monitor the quality of Stout.
- He was forced to use a pen-name by his employer-he chose to use the name Student.
- Most useful when comparing 2 sample means.
The Student t-test

• Given two data sets, each characterized by its mean, standard deviation, and number of samples, we can determine whether the means are significant by using a t-test.

\[ t = \frac{M_1 - M_2}{\sqrt{SE_1^2 + SE_2^2}} \]

A t-test is nothing more than a signal:noise ratio.

Note below that the difference between the means is the same but the variability is very different.

An Example

<table>
<thead>
<tr>
<th>Drop</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>345</td>
<td>134</td>
</tr>
<tr>
<td>2</td>
<td>376</td>
<td>116</td>
</tr>
<tr>
<td>3</td>
<td>292</td>
<td>154</td>
</tr>
<tr>
<td>4</td>
<td>415</td>
<td>142</td>
</tr>
<tr>
<td>5</td>
<td>359</td>
<td>277</td>
</tr>
<tr>
<td>6</td>
<td>364</td>
<td>111</td>
</tr>
<tr>
<td>7</td>
<td>298</td>
<td>189</td>
</tr>
<tr>
<td>8</td>
<td>295</td>
<td>187</td>
</tr>
<tr>
<td>9</td>
<td>352</td>
<td>166</td>
</tr>
<tr>
<td>10</td>
<td>316</td>
<td>184</td>
</tr>
</tbody>
</table>

• The null hypothesis states that there is no difference in the means between samples:
  1) Calculate means.
  2) Calculate SDs.
  3) Calculate SEs.
  4) Calculate t-value.
  5) Compare to ttab.
  6) Accept/reject H_0.

**Plot Data**

**Box Plot**

**Bar Graph**

**1) Calculate Mean**

\[ M_1 = \frac{\sum X_1}{N_1} = \frac{345 + 376 + 292 + 415 + 359 + 364 + 298 + 295 + 352 + 316}{10} = 341 \]

\[ M_2 = \frac{\sum X_2}{N_2} = \frac{134 + 116 + 154 + 142 + 177 + 111 + 189 + 187 + 166 + 184}{10} = 156 \]

**2) Calculate SD**

\[ SD_1 = \sqrt{\frac{\sum (X_1 - M_1)^2}{N_1 - 1}} = \sqrt{\frac{323 + 2401 + 324 + 1449 + 3336}{9}} = 80 \]

\[ SD_2 = \sqrt{\frac{\sum (X_2 - M_2)^2}{N_2 - 1}} = \sqrt{\frac{484 + 1600 + 4 + 2625 + 1089 + 1521 + 2049 + 1951 + 2891 + 2891}{9}} = 90 \]

**3) Calculate SE**

\[ SE_1 = \frac{SD_1}{\sqrt{N_1}} = \frac{40}{\sqrt{10}} = 4.0 \]

\[ SE_2 = \frac{SD_2}{\sqrt{N_2}} = \frac{29}{\sqrt{29}} = 9.1 \]
4) Calculate the t-statistic

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>341</td>
</tr>
<tr>
<td>SD</td>
<td>40</td>
</tr>
<tr>
<td>SE</td>
<td>13</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
</tr>
</tbody>
</table>

\[
t = \frac{M_1 - M_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}
\]

Now we have to compare our t-value to a table of critical t-values to determine whether the sample means differ.

What on Kronos are Degrees of Freedom?????

- Describe the number of values in the final calculation of a statistic that are free to vary.

Since we have 2 data sets, then \( df = 2(N) - 2 \)
\( df = 2(10) - 2 = 18 \)

But....

5) Compare \( t_{calc} \) to \( t_{tab} \) for 18 df

- For the 95% confidence level and a df of 18, \( t_{tab} = 2.101 \). Our t-value was 11.6.

Since \( t_{calc} > t_{tab} \), then we must reject the \( H_0 \) and conclude that the sample means are significantly different.
The P Value

- A small p-value (typically ≤ 0.05) indicates strong evidence against the null hypothesis, so you reject the null hypothesis.
- A large p-value (> 0.05) indicates weak evidence against the null hypothesis, so you fail to reject the null hypothesis.
- p-values very close to the cutoff (0.05) are considered to be marginal (could go either way). Always report the p-value so your readers can draw their own conclusions.

One-tailed vs two-tailed t-test

One-tailed t-test
- A two-tailed test will test both if the mean is significantly greater than x and if the mean significantly less than x.
- The mean is considered significantly different from x if the test statistic is in the top 2.5% or bottom 2.5% of its probability distribution, resulting in a p-value less than 0.05.

Two-tailed t-test
- A one-tailed test will test either if the mean is significantly greater than x or if the mean is significantly less than x, but not both. The one-tailed test provides more power to detect an effect in one direction by not testing the effect in the other direction.

Paired vs Unpaired t-test

Paired
- The observed data are from the same subject or from a matched subject and are drawn from a population with a normal distribution.
- Example: Measuring glucose concentration in diabetic patients before and after insulin injection.

Unpaired
- The observed data are from two independent, random samples from a population with a normal distribution.
- Example: Measuring glucose concentration of diabetic patients versus non-diabetics.

Comparing Three or More Means

Why not just do multiple t-tests?

- If you set the confidence level at 5% and do repeated t-tests, you will eventually reject the null hypothesis when you shouldn’t i.e. you increase your chance of making a Type I error.

To compare three or more means we must use Analysis of Variance (ANOVA)

- In ANOVA we don’t actually measured variance. We measure a term called “sum of squares.”
- There are 3 sum of squares we need to measure.

1) Total sum of squares.
   - Total scatter around the grand mean.
2) Between-group sum of squares.
   - Total scatter of the group means with respect to the grand mean.
3) Within-group sum of squares.
   - The scatter of the scores.
Frog Germ Layer Experiment

Germ Layer Surface Tensions

Anova/MCT

Endo vs Meso
Endo vs Ecto
Meso vs Ecto
Ecto vs Ecto under

Endo vs Ecto under

ANOVA

The fundamental equation for ANOVA is:

\[ SS_{\text{Tot}} = SS_{BG} + SS_{WG} \]

From this we can calculate the mean sum of squares by dividing the sum of squares by the degrees of freedom.

\[ MS_{BG} = \frac{SS_{BG}}{df_{BG}} \quad MS_{WG} = \frac{SS_{WG}}{df_{WG}} \]

We can then calculate the F statistic:

\[ F = \frac{MS_{BG}}{MS_{WG}} \]

To calculate sums of squares we first need to calculate two types of means.

1) Group means (\( \bar{X} \))
2) The grand mean (\( \bar{X} \))

\[ SS_{\text{Tot}} = \sum(X - \bar{X})^2 \]

\[ SS_{BG} = \sum(\bar{X} - \bar{X})^2 \times \# \text{ groups} \]

\[ SS_{WG} = \sum(x - \bar{x})^2 \]

df for ANOVA

- To calculate the MS BG and MS WG, we need to know the Df.

\[ MS_{BG} = \frac{SS_{BG}}{df_{BG}} \quad MS_{WG} = \frac{SS_{WG}}{df_{WG}} \]

- To determine the df for these two parameters we need to partition:

  - df of SS BG is n-1 of how many groups there are. Therefore for 3 groups, df=2.
  - df of SS WG is n-1 of all groups. Therefore for 30 samples (10 in each of the 3 groups), df=27.

- We can then compared the Fcalc to the Ftab to determine whether significant differences exist in the entire data set.

One-way versus two-way ANOVA

One-Way ANOVA

- 1 measurement variable and 1 nominal variable.
- For example, you might measure glycogen content for multiple samples of heart, liver, kidney, lung etc...

Two-Way ANOVA

- 1 measurement variable and 2 nominal variables.
- For example, you might measure a response to three different drugs in both men and women. Drug treatment is one factor and gender is the other.
ANOVA only tells us that the smallest and largest means likely differ from each other. But what about other means?

In order to test other means, we have to run post hoc multiple comparisons tests.

Post hoc tests
- Are only used if the null hypothesis is rejected.
- There are many, including Tukey’s, Bonferroni’s, Scheffe’s, Dunn’s, Newman-Keuls.
- All test whether any of the group means differ significantly.
- These tests don’t suffer from the same issues as performing multiple t-tests. They all apply different “corrections” to account for the multiple comparisons.
- Accordingly, some post hoc tests are more “stringent” than others.

Linear Regression
- The goal of linear regression is to adjust the values of slope and intercept to find the line that best predicts Y from X.

• More precisely, the goal is to minimize the sum of the squares of the vertical distances of the points from the line.

Note that linear regression does not test whether your data are linear. It assumes that your data are linear, and finds the slope and intercept that make a straight line that best fits your data.

$r^2$, a measure of goodness-of-fit of linear regression
- The value $r^2$ is a fraction between 0.0 and 1.0, and has no units.
- An $r^2$ value of 0.0 means that knowing X does not help you predict Y.
- When $r^2$ equals 1.0, all points lie exactly on a straight line with no scatter. Knowing X lets you predict Y perfectly.

How is $r^2$ calculated?
- The left panel shows the best-fit linear regression line. In this example, the sum of squares of those distances (SSreg) equals 0.86.
- The right half of the figure shows the null hypothesis — a horizontal line through the mean of all the Y values. Goodness-of-fit of this model (SStot) is 4.907.

$r^2 = 1 - \frac{SS_{res}}{SS_{tot}} = 1 - \frac{0.86}{4.91} = 0.83$. 
Power Analysis: How many samples are enough?

- If sample size is too low, the experiment will lack the precision to provide reliable answers to the questions it is investigating.
- If sample size is too large, time and resources will be wasted, often for minimal gain.

Calculation of power requires 3 pieces of information:

1) A research hypothesis.
   - This will determine how many control and treatment groups are required.
2) The variability of the outcomes measure.
   - Standard Deviation is the best option.
3) An estimate of the clinically (or biologically) relevant difference.
   - A difference between groups that is large enough to be considered important. By convention, this is set at 0.8 SD.

An Example

- We would like to design a study to measure two skin barriers for burn patients.
- We are interested in "pain" as the clinical outcome using the "Oucher" scale (1-5).
- We know from previous studies that the Oucher scale has a SD of 1.5.

Here's the equation:
\[
 n = \frac{(\sigma_1^2 + \sigma_2^2) \times (z_{1-\alpha/2} + z_{1-\beta})^2}{D^2}
\]

Here, \( z_{1-\alpha/2} \) is the critical value of \( z \) at 0.975 (1.96) and \( z_{1-\beta} \) is power at 80% (0.84).

What would happen if our clinically relevant difference was set at 1 Oucher unit? Here:
\[
 n = \frac{(1.5^2 + 1.5^2) \times (1.96 + 0.84)^2}{1^2} = 35.3 = 36
\]

What would happen if our clinically relevant difference was set at 0.5 Oucher units? Here:
\[
 n = \frac{(1.5^2 + 1.5^2) \times (1.96 + 0.84)^2}{0.5^2} = 141.12 = 142
\]

Another Example

- You want to measure whether aggregates of invasive cell lines are less cohesive than those generated from non-invasive counterparts.
- We know that SD for the control group is 3 dynes/cm and for the invasive group is 2 dynes/cm.
- You set the \( \alpha \) at 0.05 (=1.96) and \( \beta \) at 80% (=0.84) and D at 2 dynes/cm.
- How many aggregates from each group would you need?

Here's the equation:
\[
 n = \frac{(3^2 + 2^2) \times (1.96 + 0.84)^2}{2^2} = \frac{(9 + 4) \times (2.80)^2}{4} = \frac{13 \times 7.84}{4} = 101.9 = 25.5 = 26
\]

Therefore, we need 26 aggregates in each group to be able to reliably detect a difference of 2 dynes/cm cohesive between invasive and non-invasive cells.
In general, how do variability, detection difference, and power influence n?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>More variability in the data</td>
<td>Higher n required</td>
</tr>
<tr>
<td>Less variability in the data</td>
<td>Fewer n required</td>
</tr>
<tr>
<td>Detect small differences between groups</td>
<td>Higher n required</td>
</tr>
<tr>
<td>Detect large differences between groups</td>
<td>Fewer n required</td>
</tr>
<tr>
<td>Smaller ( \alpha ) (0.01)</td>
<td>Higher n required</td>
</tr>
<tr>
<td>Less power (smaller ( \beta ))</td>
<td>Fewer n required</td>
</tr>
</tbody>
</table>

Prism Stats Guide

- [http://www.graphpad.com/welcome.htm](http://www.graphpad.com/welcome.htm)
Investigating Interactions of Mammalian DNA Double Strand Break Repair

Sarah Misenko
18 October 2013

DNA Damage and Repair Pathways

Double Strand Break Repair

BRCA1 and Cancer

Targeted Cancer Therapies

Bloom’s Helicase

- Bloom’s Syndrome:
  - Short stature
  - Skin sensitivity
  - Predisposition to cancer
- Investigating the function of BLM:
  - Interact with the Rif1/53BP1 complex to regulate DSB repair pathways?
  - Mediate DNA resection
  - Play a role in the disassociation of RAD51 foci?

- Drugs that block the growth of cancer by targeting the cellular changes in the cancer cells
- BRCA1 deficiency is synthetically lethal when PARP is inhibited
BLM and BRCA1/BLM deficient cells show higher frequencies of radial chromosomes, a marker of defective HR.

BRCA1, BLM, and BRCA1/BLM double mutants showed higher aberration frequencies than WT cells.

BLM deficient cells show increased rates of SCE.

BRCA1/BLM deficient cells show decreased SCE compared to BLM deficient cells. This suggests there may be a defect in HR.

• Measure DNA resection in BLM deficient cells
• Determine effect of RAD51 depletion in BLM deficient cells
• Determine genomic stability of BLM deficient cells with various DSB repair gene deficiencies (ex. 53BP1 and EXO1)

• QUESTIONS?
Engineering TPP1 variants with improved therapeutic properties for effective enzyme replacement therapy in Late Infantile Neuronal Ceroid Lipofuscinosis (LINCL)

Narendra Kuber
3rd year, GSBS

ACKNOWLEDGEMENT

Principal Investigator:
Dr. Peter Lobel

Associate Professor:
Dr. David Sleat

All lab members

Special thanks to:
Dr. Vik Nanda for advice on protein design
Dr. Ann Stock for her contribution in resolving the crystal structure
Committee and staff of the Biotech Training Program

NIH funding for the Biotechnology Training Program

Lysosomal Storage Disorder

- pH ~ 5.0
- Degradation and recycling center

- Accumulation of undigested material
  - Deficient enzyme
  - Mutations in membrane proteins

Assocations of mutations in a lysosomal Protein with Classical Late-Infantile Neuronal Ceroid Lipofuscinosis. Sleat et al., Science, Sept. 1997, 1802-1805

- CLN2 codes for TPP1
- The missing spot at ~46kD and pI of ~pH 6 is TPP1
- Sequenced and characterized

Pro TPP1 transport and autocatalysis

JBC Vol. 284, NO. 6, 3985-2997, Feb 2009
Guhaniyogi et al.
**Modalities of treatment**

- Small molecule chaperone therapy
  - Function present to some degree
- Gene therapy
  - Promising yet some concerns
- Enzyme replacement therapy
  - Intravenous (IV)
  - Intrathecal
  - Intraventricular

**Hypothesis**

Stabilizing TPP1 structure through one or more mutations will result in a longer intra-lysosomal half-life that will increase survival rates and reduce accumulated substrate.

**Strategies to stabilize TPP1 structure**

- Secondary structures
  - X x x → Gly at α-position
  - X x x → Pro to stabilize surface exposed loops

- Tertiary folds
  - Cysteine pairs for disulfide bond formation
  - Charged residues to cohere salt bridges
  - Kumarnolysin as a guide

- Post-translational modifications
  - Introduction of N-glycosylation sites (N-x/S/T)
  - Increase in half-life by decreasing the population of protease susceptible unfolded state and aggregation prone misfolded state

**Engineering of TPP1 variants**

- Introduce desired mutations in human TPP1 cDNA clone
- Transient transfection in CHO cells for variant expression
- Half-life measured in LINCL lymphoblasts
- $K_{cat}$ and $K_{m}$ calculation
- Acute and chronic dosing
- Superiority to wild-type TPP1

**Overview**

- Computational structure-based design of TPP1 mutations to increase stability
- Engineer TPP1 variants and express in CHO cells
- Be able to measure activity, half life and characterize pharmacokinetic properties
- Test potential drug candidate in a mouse model
Quantitative assay for measuring TPP1 activity

- The fluorescence can be read in a fluorometer at excitation wavelength 360/20 nm and emission wavelength of 460/25 nm
- This assay can be used two ways: kinetic mode to calculate the rate of reaction and end-point mode to determine half-life

Rules for designing disulfide bonds

Design of Disulfide-linked Thioredoxin Dimers and Multimers Through Analysis of Crystal Contacts

Results and Discussion

Other criteria listed are:
- Residues should not be a part of the catalytic site
- Residues should not be involved in any secondary (side-chain to main chain hydrogen bonding) or tertiary hydrogen bonding interactions
- The introduced disulfide bond should have good stereochemistry
- Atypical stereochemical features would result in higher strain energy that can offset the stabilizing effect

Ramachandran plot of residues in alpha-left conformation

Residues H511, E532, S369, W470 and R465 mutated to Gly

Strategy for designing Xxx → Pro mutations

- Identify conserved proline residues in mature form of TPP1 and kumamolysin
- Inspect regions between two consecutive conserved Pro residues in TPP1 and compare with Kumamolysin
- Decision to introduce proline was made based on:
  - Positions of existing amino acid residues on the Ramachandran plot favoring Pro substitution

N-Glycosylation can increase the rate of protein folding, enhance thermodynamic stability, and slow protein unfolding; however, the molecular basis for these effects is incompletely understood. Without clear engineering guidelines, attempts to use N-glycosylation as an approach for stabilizing proteins have resulted in unpredictable energetic consequences. Here, we review the recent development of three “enhanced aromatic sequons,” which appear to facilitate stabilizing native-state interactions between Phe, Ala-Glu-Nac, and Thr when placed in an appropriate reverse turn context. It has proven to be straightforward to engineer a stabilizing enhanced aromatic sequon into glycoprotein-native-like proteins that have not evolved to optimize specific protein-carbohydrate interactions. Incorporating these enhanced aromatic sequons into appropriate reverse turn types within proteins should enhance the well-known pharmacokinetic benefits of N-glycosylation-based stabilization by lowering the population of protease-resistant unfolded and aggregation-prone misfolded states, thereby making such proteins more useful in research and pharmaceutical applications. # 2012 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 98: 195–211, 2012.
Kumamolysin can serve as a template for mutating TPP1

30% sequence identity between Kumamolysin and TPP1

Mutants ready for characterization

<table>
<thead>
<tr>
<th>Type</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys-Cys double mutant</td>
<td>D457C-S472C</td>
</tr>
<tr>
<td>Xxx → Gly</td>
<td>H511G_Kz</td>
</tr>
<tr>
<td>Xxx → Gly</td>
<td>E532G_Kz</td>
</tr>
<tr>
<td>Xxx → Gly</td>
<td>S369G_Kz</td>
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<td>Xxx → Gly</td>
<td>W470G</td>
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<td>Xxx → Gly</td>
<td>R465G_Kz</td>
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Arthur D. Casciato (Ph.D., University of Virginia) is the Director of the Office of Distinguished Fellowships. He came to Rutgers in fall 2007 from the University of Pennsylvania where he was adjunct associate professor of English and founding Director of the Center for Undergraduate Research and Fellowships. Before his appointment at Penn, he was an associate professor of English and American Studies at Miami University of Ohio. He has co-edited two books, Waiting for Nothing and Other Writings by Tom Kromer, and Critical Essays on William Styron, as well as published articles on the novels of Styron, Theodore Dreiser, John Barth, and Pietro Di Donato. He was also the co-founder and first book review editor of the journal, American Literary History.

The Office of Distinguished Fellowships, led by Dr. Arthur Casciato, offers assistance in all stages of the application process for major national fellowships and postgraduate support. We provide information on available programs, strategic planning, advice, and support for completing a competitive application. Through this office, currently enrolled and recently graduated students can learn about the many fellowships that are available through governments, private donors, foundations, civic organizations, and corporations. Faculty referrals and participation in the process are vital for an applicant’s initial involvement and success.

This office is geared not only for students in their last year of college, but for all students—from first-years to seniors—who want to pursue their academic goals beyond Rutgers through a national fellowship. All students are encouraged to take advantage of the resources of this office and learn about these fellowships early in their college careers. Likewise, faculty members are requested to recommend promising students of any year with strong academic records and breadth of experience to this office.
Development of a topical dual-cell therapy for wound healing

Paulina Krzyszczyk
11/1/2013

Wound Statistics

- Chronic Wounds
  - 6.5 million people in the U.S.
  - Pressure, venous and diabetic ulcers
- Diabetic ulcers
  - 25.8 million diabetics in the U.S.
  - 15-25% diabetics with foot ulcers
  - 60% non-traumatic lower-limb amputations
- Burn Injury
  - 11 million people treated for burns (2004)
  - Excessive leftover scarring and contracture

Challenges with Chronic and Burn Wounds

- Risk of infection
- Dysfunctional inflammatory phase
  - ↑ Number of immune cells
  - ↑ Protease levels
  - ↓ Growth factors

Clinical Treatments and Their Shortcomings

- Primary goal—achieve wound closure
- Debridement—removal of necrotic tissue
- Wound dressings—maintain a moist wound environment
- Auto/allografting—creates a secondary wound site
- Bioengineered tissues—low cell viability, scarring, altered mechanical properties

Current Research Areas in Wound Healing

- Cell therapies
  - Endothelial cells, melanocytes, stem cells
  - Genetically modified cells
  - Microencapsulated cells
- Biological factors
  - Vascular endothelial growth factor (VEGF)
  - Epidermal growth factor (EGF)
  - Basic fibroblast growth factor (bFGF)
  - Insulin
- Tissue Engineering Scaffolds
  - Decellularized xenogenic tissue
  - 3D-printed
  - Electrospun

Promise of Insulin for Wound Healing

- Insulin
  - peptide hormone
  - pancreatic beta cells
  - plays a role in wound healing
  - Affects keratinocytes, fibroblasts and endothelial cells
- In vivo, topical insulin has accelerated
  - angiogenesis
  - reepithelialization
  - wound closure
- Limitations are
  - Frequent topical applications
  - "One size fits all" dosing
Slide 1

P1 Is the title too broad? Should it be more specific, including the cell types and mention of encapsulation method?
Paulina, 10/27/2013

Slide 5

P2 Change title to Current Research Trends in Wound Healing?
Paulina, 10/30/2013
Promised of Mesenchymal Stem Cells (MSCs) for Wound Healing

- MSCs are involved in all phases of wound healing
  - Regulate cytokine production and immune response
  - Produce growth factors (VEGF, HGF, PDGF-BB)
  - Possess anti-scarring properties
  - Control collagen deposition
- Accelerated healing in clinical trials
- Limitations
  - Loss of cells due to migration or cell death
  - Internalization and varying degrees of immune response

Proposed Treatment

- Co-encapsulation of insulin-producing beta cells and MSCs in microspheres for use as a topical wound healing therapy
- poly(ethylene glycol) diacrylate (PEGDA)
  - FDA-approved
  - Non-immunogenic
  - Immuno-protective
  - Photo-polymerizable with white light
- Hypothesis: Therapy will result in sustained release of insulin and growth factors directly to wound site to accelerate healing

Current Work

- Evaluating the viability of encapsulated cells
- Detect the secreted factors using ELISA
  - Insulin
  - Insulin growth factor-1 (IGF-1)
  - Platelet-derived growth factor-BB (PDGF-BB)
  - Granulocyte colony-stimulating growth factor (G-CSF)
  - VEGF, HGF, KGF
- Pilot study—in vivo diabetic mouse wound model + encapsulated beta cells

Future Steps

- Encapsulate beta cells and MSCs in separate microspheres
- Co-encapsulate beta cells and MSCs in same microspheres
  - Vary the ratio between the two cell types in order to optimize cell viability and secretion of growth factors
  - In vivo mouse and pig wound models

References


Acknowledgements

- Biotechnology Training Program
- Dr. Ronke Olabisi
- Dr. François Berthiaume
- Ayesha Aijaz
- Olabisi Lab Members
- Mehdi Ghodbane
- Andrea Gray

Questions?
An In Vitro Alternative to Replace Animal Testing to Identify Skin Allergens

Seron Lee
Dr. Martin Yarmush
Biotech Presentation
11 – 1 - 2013

Allergic Contact Dermatitis

Most commonly reported occupational hazard

Annual costs >$1 billion

~3000 contact allergens identified

Traditional screening assays utilize animal testing to assess risk and limit public exposure

Sensitizers

1. Haptens (Eg. Metals)
   - Low molecular weight (<1000 Da)
   - Lipophilic (log P ~2)
   - Electrophilic

2. Pre-Haptens: (Eg. Dyes, Fragrances)
   - Auto-oxidation
   - Non-Sensitizer
   - Sensitizer
   - ~30-50% of all sensitizers

3. Pro-Haptens: (Eg. Fragrances)
   - Metabolism
   - Non-Sensitizer
   - Sensitizer

Mechanism

Antigen (PPD) specific T-Cell Response on Skin

Current Methods and Limitations

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<th>Specificity</th>
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<td>In Vitro</td>
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<td>Medium</td>
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<tr>
<td>In Silico</td>
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<td>High</td>
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<tr>
<td>In Chemico</td>
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- Poor assay performance detecting pre-/pro-hapten sensitizers across current alternatives
- Most prominent in silico and peptide binding assays when there is no metabolic component

Hypothesis

Motivation

1. Current alternatives to animal tests lack a sufficient metabolic component to accurately identify pro-hapten sensitizers.

2. Current alternatives must be capable of performing high throughput screens of compounds with unknown sensitization potentials.

Hypothesis

An in vitro culture system integrated with a metabolic component can be used to identify pro-hapten sensitizers in a high throughput fashion.

End Goal

A high-throughput screening assay that accurately predicts the sensitization potential of chemicals as an alternative to animal testing
**Human Liver Microsomes**

- Contains Phase I (CYP) and Phase II (UGTs) enzymes
- Commonly Used in DMPK Studies
- High throughput
- Cost Effective

Co-incubation of THP-1 cells and human liver microsomes (S9 fraction) enhanced CD86 fluorescence intensity.

**Cyp 1A1/1A2 Activity**

Cyp1A1/1A2 Activity for Human Liver Microsomes (HLM) was equivalent to HepG2 cells.

**Chipinda et al. Toxicology 2011**

**Cyp 1A1/1A2 Activity**

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**MutzLC HepG2 HLM**

- CYP1A1/1A2 Activity

**Cyp1A1/1A2 Activity**

1 mg/mL HLM (s9)

NADP

isocitric acid

*Resorufin

$\text{t=0-90min}$

$N=6, P < 0.05$ by Anova with Fisher LSD post-hoc analysis

**Cyp1A1/1A2 Activity**

- Enhanced expression of CD86 for pre-/pro-hapten PPD with microsomes

**Enhanced expression of CD86 for pro-hapten isoeugenol with microsomes**

**Summary**

- Determined Mutz-LCs are insufficient source for metabolic conversion of pro-haptens
- Demonstrated higher metabolic activity in microsomes than Mutz-LCs (CYP1A1)
- Microsomes successfully activated PPD and IE to induce enhanced Mutz-LC maturation response (CD86)

**Future Work**

- Optimize concentration of microsomes and incubation time
- Characterize metabolic activity of additional CYP enzymes
- Evaluate additional cellular metrics of sensitization (cytokines, CD54, etc.)
- Expand panel of test chemicals
Questions

Committee:
Dr. Martin Yarmush
Dr. Rene Schloss
Dr. Bozena Michniak-Kohn
Dr. Timothy Maguire
Dr. Miri Sieberg

Yarmush Lab
Berthiaume Lab
Michniak-Kohn Lab

Biotechnology Training Program
The Role of Bloom’s Helicase in Mammalian DNA Double Strand Break Repair

Bharm Patel
NJ Biotechnology Training Program
Bunting Lab
November 15th, 2013

Hallmark of Cancer: Genomic Instability
Accumulation of genomic alterations may cause dys-regulation of cell division, imbalance between cell growth and death, and cancer.

Bloom’s Syndrome and BLM Helicase
Bloom syndrome is an inherited disorder characterized by short stature, sun-sensitive skin changes, an increased risk of cancer, infertility, and other health problems.
- Low birth weight and height
- Patches/Blotchy pigmentation of skin, especially in sunlight
- Predisposition to the early development of cancers
- 205 reported cases through 2009
- Carrier Frequency: 1 in 110

Mutations in blm are responsible for Bloom’s Syndrome
- Belongs to RecQ family of helicases
- 3’ to 5’ helicase activity and requires ATP
- 5 RecQ helicases in humans

Homologues:
- S. cerevisiae homologue: Sgs1
- S. pombe homologue: Rgh1
- E. coli homologue: RecQ

Sgs1 acts to suppress crossing-over following DSB (Ira 2003)

WT Blm -/-
10x Increased SCEs

Functions of BLM Helicase
Mechanistic aspects of Blm Helicase:

DNA Damage
DNA damage can occur due to:
1. Replication fork collapse
2. Endogenous chromosome damage
3. Ionizing radiation
4. Mutagenic chemicals
5. Programmed recombination events (ex. Meiosis, Ab diversity)

Results in:
1. Proper repair (NHEJ and HR)
2. Cell cycle arrest
3. Transcriptional activation of repair genes/apoptotic genes
4. Cell death!

DNA Repair Pathways - NHEJ vs HR
Collaboration of BRCA1 loss and 53BP1 loss results in synthetic viability and restoration of HR-mediated repair.

**Project**

**Objective:** Understand the specific function of BLM in DNA resection and overall genomic stability in mammalian cells.

**Hypothesis:** Defect in HR in BLM deficient mammalian cells can be compensated by depletion of 53BP1.

**Rationale:** Loss of 53BP1 is able to reverse the HR defective phenotype associated with BRCA1 loss (synthetic viability).

**Specific Aims:**
1. Generate an animal model to study the genetic interactions of proteins involved in HR and NHEJ DSB repair pathways by breeding mice with single and combined deficiencies in BRCA1, BLM, Exo1, and 53BP1.
2. Elucidate the role and mechanism of action of BLM in DNA resection by identifying sensitivity to replication stress and DSB causing agents.
3. Identify genetic interactions between proteins involved in HR and NHEJ to provide cytotoxicity to cancer cells in which DNA repair defects have been identified.

**Why is studying BLM important?**

Link between DNA helicase gene mutations and cancer-predisposing diseases, such as Bloom’s Syndrome and Werner’s Syndrome.

Inactivation of specific DNA helicases provides new avenues of exploiting synthetic lethality to provide cytotoxicity to cancer cells in which DNA repair defects have been identified.

DNA helicase overexpression in cancer cells provides resistance to chemotherapy treatments and thus, may represent a biomarker for response to chemotherapy in HR-defective tumors.

At present, the functions of BLM in mammalian DNA repair are poorly defined. Mechanistic details will provide insight into possible clinical applications to combat cancers with personalized medicine.
SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients

Trevan Locke

PMDS background

- Phelan-McDermid Syndrome is a gene disorder resulting from the deletion of a portion of chromosome 22.
- Characterized by developmental delay, intellectual impairment, and often autistic-like behavior.
- Almost all cases involve deletion of SHANK3, a gene that encodes a scaffold protein that connects membrane bound receptors to the actin cytoskeleton.

PMDS patient selection

Differentiation

Removal of growth factors and induction of neuronal fate

Introduction of fluorescent proteins through lentiviruses

Introduction of neuronal medium followed by isolation of rosettes.

PMDS and control cells co-cultured on astrocytes

Neuronal Markers present in differentiated cells

Synapse Review

- Post-synaptic density
  - PSD, concentration of receptors and other proteins at the post-synaptic membrane
- EPSC makes cell more likely to fire action potentials
- IPSC makes cell less likely to fire action potentials
- Puncta clusters of proteins within PSD, show up as bright dots with fluorescent imaging
PMDS Neuron Characterization

- PMDS neurons showed increased input resistance and decreased amplitude and frequency.
- Similar action potential, resting membrane potential, and capacitance

Inhibitory synaptic transmission was identical to control neurons

SHANK3 Expression

- As mentioned, PMDS neurons have decreased levels of SHANK3 expression.

SHANK3 Expression

- Transduced SHANK3 lentivirus restores AMPA-EPSC's in all cells and NMDA-EPSC's in 43% of cells

Treatment with IGF1 restores deficits
IGF1 Treatment

• Difference between SHANK3 and IGF1 deficit restoration
• IGF1 actually reduces SHANK3 expression, but compensates by increasing PSD95 expression
• Changes in NMDA decay rate indicative of this difference

Summary

• Loss of SHANK3 implicated in onset of PMDS.
• Patient derived iPSC’s reprogrammed to neurons to study gene expression and synapse activity.
• PMDS neurons have decreased excitatory transmission.
• Transduced SHANK3 and administered IGF1 are shown to recover synaptic deficits.

Critique

• Controls lacking in SHANK3 transduction experiments.
• There were only two patients used.
• Co-culture model allows control and PMDS cell lines to be studied under identical conditions in addition to showing cell autonomy.

Acknowledgements

• Dr. Bonnie Firestein
• Ana Rodriguez
• Ilija Melentijevic
• Rutgers Biotechnology Training Program

AdditionalReferences


Questions?
Who owns your body?

HeLa Cells

- Immortal cell line
- Reproduce in 24 hours
- First human biological materials to be bought and sold

1951: Removed from Henrietta Lacks by Howard Jones, MD
Given to George Gey, MD at JHU

1952: Used by Jonas Salk to isolate first polio vaccine

2009: Over 60,000 research papers studying
- AIDS
- Cancer
- Cloning
- Gene mapping
- Vaccines
- Toxicity
- ...
1974: Researchers worldwide discovered that HeLa cells had migrated to contaminate other cultures.

1975: Family contacted for tests to distinguish HeLa from other cells. Family told tests were to see if the cancer was hereditary.

1976: Hairy cell leukemia cells taken from John Moore. Patented cell line used to study:
- Leukemia
- Anti-cancer drugs
- Crohn's disease
- Granulocyte-macrophage colony-stimulating factor

Mo Cells

Estimated market value: $3,000,000,000

- HeLa:
  Q1) Informed consent: Ms. Lacks was informed of her treatment. What is wrong with using something that someone else has thrown away?
  Q2) Doesn't Ms. Lacks own her own cells?
    - if yes, can't the state declare eminent domain?
    - if no, why do we need donor cards?
  Q3) What about the profits from Ms. Lacks' cells?

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

- Ruth Faden, JHU
Q1) Since UCLA lied about the uses of Moore’s cells on his informed consent form, isn’t the consent meaningless? Would Moore’s control over his cells have a “chilling effect” on biomedical research?

Q2) Should Moore have right to some of the profits derived from his cells? What about the value added by UCLA and other researchers?

Beleno v. Texas (also Bearder v. Minnesota)

- DNA tests on 4.5 million newborns without consent
- Blood samples sold, sent to DNA Identification Lab

Washington v. Catalona

Dr. Catalona collected cells to create a bio-repository while at WUSTL …

… then he joined Northwestern University.

“Bill Catalona wants to send nearly 2,000 documented samples to [Hybritech] for free. Just from a cost recovery scenario, this should be worth nearly $100,000 to the university. The only consideration [Hybritech] is offering is the potential for Catalona to get a publication. It is my opinion this is an unacceptable proposal.”

Who owns the samples?

The patients?

Dr. Catalona?

Northwestern University?

WUSTL?

Who owns the eggs?

Mitochondrial diseases:

- Alpers disease
- Complex I deficiency
- Complex III deficiency
- Complex IV deficiency
- Chronic Progressive External Ophthalmoplegia Syndrome
- Kearns-Sayre Syndrome
- Long-Chain Hydroxyacyl-CoA Dehydrogenase deficiency
- Leber Hereditary Optic Neuropathy
- Myoclonic Epilepsy
- Ragged-Red Fiber Disease
- Retinitis Pigmentosa
...
A Tale of Two Tropomyosins: How protein structure and stability correlates with allergic exposure in food

Jose James
Nanda Lab

Clinical Data on Food Allergy

Only very selective types of food induce an allergic response
Ingestion of allergen is the primary method to induce an allergic reaction

Immunodominant allergens are proteins

What determines if a protein is a potential antigen??

Shrimp TM withstands digestion in gastric fluid

Simulated Gastric Fluid: a buffered solution of pepsin at pH 2

Shrimp and pig TM have distinct denaturation profiles

-sTM shows a single, global unfolding transition, indicating a highly cooperative and stable molecule
-pTM denaturation shows multiple transitions including unstable and stable domains
Can we understand sTM allergenicity in terms of its structure?

Hypothesis – effective survival of TM epitopes is dependent on the stability of the corresponding sequences against digestion from proteases to allow immune exposure distally in the GI tract.

Predictions:
(1) Regions that survive simulated gastric and intestinal digestion with residual structure align with those found clinically as allergic epitopes.
(2) By changing the stability of shrimp and pig TM we can alter the survival of these regions.

Strong overlap exists between fragment survival in simulated digestion and known allergic epitopes.

Survival scores based on relative abundance during early and late digestion

MD shows significant correlation between low micro-unfolding events in epitope regions and survival probability based on number of micro-unfolding events per residue combined with the locations of pepsin, trypsin, chymotrypsin cleavage sites.

Conclusions:
- We have observed two digestion phenotypes for TM:
  - sTM - rapid digestion of the NTH is followed by slow digestion of the CTH
  - pTM - digestion is rapid and no persistent high MW species are observed.
- sTM and pTM display differences in unfolding. Regions that persist during digestion show a more robust α-helical structure with minimal susceptibility to micro-unfolding.
- Position 126 may play a crucial role for tuning the global stability of TM, thereby altering its digestion properties.

Future directions:
- Swapping digestion-phenotypes of sTM and pTM
  - Generate mutant sTM-M126G and pTM-G126M
  - Determine if mutants have an altered digestion profile resembling its counterpart. Will Pig be more like Shrimp and vice versa?
  - Understand how G126 affects the global stability of TM
- Survey TM other animal sources
  - Do TM from other vertebrates and invertebrates show the same digestion phenotype differences as pTM and sTM?
Acknowledgements

- Thanks to Dr. Nanda and the Nanda lab!!!
  - Especially Douglas Pike and I. John Khan

- Funding:
  - NIH
  - Rutgers Biotechnology Program
Multiresponsive liposomes with pH-triggered targeting and content release for cancer chemotherapy

Michelle Sempkowski
Advisor: Dr. Stavroula Sofou
12/06/2013

Motivation

- Early detection has reduced cancer death rates, but there is still no cure for the disease in its advanced stage
- Critical barriers:
  1. Low tumor targeting selectivity with poor drug uptake by cancer cells
  2. Heterogeneous distribution of the drug within the tumor
  3. Insufficient drug bioavailability within cancer cells comprising the tumor combined with high accumulation of the therapy in normal tissue (toxicity)
- Liposomes encapsulating doxorubicin are a promising approach to cancer chemotherapy, but existing approaches fall short
  - Doxil®/Caelyx®
  - Thermodox®
  - Functionalized liposomes

Our Hypothesis

Liposomes that address simultaneously all the aforementioned critical barriers will have a better therapeutic index

Our Objective

To combine all properties in a single targeted liposome system and show additive or synergistic efficacy

Rationale

Simple biophysical principles and endogenous triggers can be used to address realistic problems
Preliminary Combination Liposome

- Combination anti-HER2 liposomes with DXR
- Single property anti-HER2 binding liposomes with DXR
- DSPC/Cholesterol anti-HER2 liposomes with DXR
- Free doxorubicin
- Empty anti-HER2 and non-targeted liposomes

Rutgers

pH triggered mechanism

- pH-triggered mechanism
  - pH-controlled electrostatic repulsion in H-bonding
  - Domain formation increases local multivalency (effective avidity?)

Rutgers

Tuning membrane’s effective pKa enables fine tuning of masking/unmasking

- Phosphatidyl serine (PS) titratable headgroup
- Phosphatidic acid (PA) titratable headgroup

Rutgers

pH triggered mechanism

- Environmentally responsive surface topography (functionality)
- % change in spherical volume
  - DSPC/Cholesterol non-targeted liposomes with DXR
  - Single property non-targeted release liposomes with DXR
  - Single property anti-HER2 release liposomes with DXR
  - Combination non-targeted liposomes with DXR
  - Combination anti-HER2 liposomes with DXR

Rutgers

Domain formation increases local multivalency (effective avidity?)

- Phosphatidyl serine (PS) titratable headgroup
- % change in spherical volume
  - 21PC:DPPS(70:30); 13% PEG
  - 21PC:DPPC(70:30); 13% PEG
  - 21PC:DPPS:DSPS (70:20:10); 13% PEG
  - 21PC:DPPS:DSPS (70:10:20); 13% PEG
  - 21PC:DPPS:DSPS (70:0:30); 13% PEG
Summary

- Preliminary data for combination liposomes show promise for increasing therapeutic index
- Systematic studies allowing us to better understand the membrane indicate that:
  - Masking/unmasking can be controlled by membrane effective pKa
  - Domain formation increases local multivalency, and thus, cell binding
- May be useful for tumors with low or variable receptor expression
- (Future) potential for a novel therapeutic modality that is applicable to a number of chemotherapeutics and targeting moieties to increase life expectancy and improve patient quality of life

Acknowledgements

- Dr. Stavroula Sofou
- Dr. Amey Bandekar
- Members of Sofou Lab
- Dr. Vassiliki Karantza (UMDNJ/CINJ)
- Biotechnology Training Program
- GAANN Fellowship
- American Cancer Society Research Scholar Grant
- NSF DMR 1207022

Questions?

pH Gradient in BT474 Spheroids (~300 µm)

Distance from spheroid center (µm)

Active loading of doxorubicin: ammonium sulfate gradient
TOPICS IN ADVANCED BIOTECHNOLOGY II  
16:125:604

<table>
<thead>
<tr>
<th>TOPIC</th>
<th>DATE</th>
<th>FACULTY MEMBER</th>
<th>STUDENT COORDINATOR</th>
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<td>Glial Scar</td>
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<td>Kathryn Drzewiecki</td>
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<td>Vik Nanda</td>
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<td>Agnes Yeboah</td>
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<td>Jose James</td>
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<td>Utilizing Stem Cells to Model Brain Disorders</td>
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<td>Bonnie Firestein</td>
<td>Ana Rodriguez</td>
<td>Ilija Melentijevic</td>
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<td>Trevan Locke</td>
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<td>Mechanobiology</td>
<td>May 2</td>
<td>Prabhas Moghe</td>
<td>Perry Yin</td>
<td>Michelle Sempkowski</td>
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<td>Pauline Krzyszczyk</td>
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Coordinators should contact the assigned faculty advisor in order to identify two papers, at least 3 weeks prior to the session. Papers are then sent to the presenters and Mary Ellen, and a first meeting is called to thoroughly review the papers (with the coordinator and the presenters).

If necessary, the group will meet with the faculty member to answer any remaining questions.

Each student prepares a 20 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 Glial Scar”

Faculty: David I. Shreiber
Students: Ileana Marrero-Berrios, Kathryn Drzewiecki

**Topics in Advanced Biotechnology**

**Neuronal Distress**

**Axonal Pathology**

**Biomaterial approaches to overcome the glial scar**

**GFAP (Glial fibrillary acidic protein) Immunohistochemistry**

**Probe design for brain-machine interfaces**

**Secondary injury cascade**

In vitro models of reactive astrocytes

Primary cortical astrocytes

Primary cortical astrocytes + Dibutyryl-cAMP

Quieting down reactive astrocytosis

Induce astrocytes into reactive phenotype with lipopolysaccharide (LPS)

No LPS

LPS + Plated MSCs

TNF-α ELISA

LPS

LPS + Encapsulated MSCs

Astrocytes and me

• Astrocytes, particularly reactive ones, are prominent in my lab’s research program

• Most of the time, we are trying to decrease or counter the influence of their reactivity

• If reactive astrocytes are so bad, why are they there in the first place? (Or said another way, what about them is good?)

• Good topic for Topics!
1. Oligodendrocyte
2. Axon Hillock
3. Neuronal cell Body
4. Myelin sheath
5. Microglia
6. Astrocyte
7. Synapse
8. Vessel from microcirculation

Why are they called glia?

Normal Functions of Astrocytes

Tripartite Synapse

Astrocyte response to damage and disease
Astrocyte response to damage and disease

The Glial Limitans

The role of the scar during CNS repair

Growth inhibition in the CNS

In vitro model of a "glial scar"
Potential mechanisms of CSPG-mediated axon growth inhibition

- A: Direct inhibition via binding to phosphatase receptors
- B: Binding to NoGo receptors (myelin-associated growth inhibitors)
- C: Converting chemorepulsive molecules
- D: Blocking growth promoting integrin binding
- E: Forming a network of inhibitory matrix

Overcoming growth inhibition

Chondroitinase ABC
Xyloside

Overcoming growth inhibition

Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury

Two Faces of Chondroitin Sulfate Proteoglycan in Spinal Cord Repair: A Role in Microglia/Macrophage Activation

Presented by Ileana

Presented by Kathryn
Two Faces of Chondroitin Sulfate Proteoglycan in Spinal Cord Repair: A Role in Microglia/Macrophage Activation

Rolls et al., PLoS Medicine 2008

Kathryn Drzewiecki
1/24/14

• Major constituent of glial scar are the chondroitin sulfate proteoglycans (CSPGs)
  1. Shown to block axon regeneration
  2. Used chondroitinase ABC to degrade CSPG

Motivation/Background

• CSPGs generally associated with wound healing

Background

• Microglia/macrophages found in the glial scar have been found to take on different phenotypes

Hypothesis

• Microglia/Macrophages can take on different phenotypes based on different stimuli
• CSPGs generally associated with wound healing

Hypothesis: Following injury, CSPG presence in the glial scar may regulate the local immune response

Methods

• Mice
  – Wild-type (WT) C57B1/6J
  – Chimeric (C) C57B1/6J – CX3CR1GFP/+:
  • Bone-marrow cells (blood-borne monocytes) express GFP
  • Resident microglia do not

• Spinal Cord Injury
  – Laminectomy at T12
  – Force on the cord of 200 kdyn

Are microglia/macrophages associated with CSPG in the lesion site?

• Mice killed 14D after spinal cord injury
• Spinal cords were fixed, dehydrated, sliced, and stained for:
  – CSPG – CS-66
  – IGF-1 – neurotrophic factor for survival
  – IB-4
  – BDNF
  – Macrophages from chimeric mice express GFP

Figure 1 Experiment
CSPG and IGF-1 expression are regionally co-localized. CSPG is co-localized with macrophages outside of the lesion.

Does CSPG activate microglia/macrophages? Does CSPG bring blood-borne macrophages to the lesion site?

- Xyloside treatment immediately followed injury
  - Injected intraperitoneally
  - Inhibitor of CSPG synthesis
- Mice killed 14D after spinal cord injury
- Spinal cords were fixed, dehydrated, sliced, and stained for CS-56, IGF-1, IB-4, GFP (blood-borne macrophages)

Xyloside treatment reduced CSPG expression halted IGF-1 expression Blood-borne macrophages infiltrated center of lesion Increased IGF-1 and spatial organization of microglia/macrophages is related to CSPG presence

Does CSPG play different roles in acute and subacute phases after spinal cord injury?

- Acute phase: CSPG → neuroprotective phenotype of macrophages/microglia
- Subacute phase: CSPG → inhibit axonal growth

Recovery scored by Basso mouse scale
- 0 (complete paralysis) to 9 (normal mobility)
- Used anterograde tracer 60D after injury with anterograde tracer biotin dextran amine
- Immunohistochemically stained for GFAP, IB-4, and CS-56
Figure 4
Restricting CSPG synthesis to the acute phase increased functional recovery

Figure 4
Following xyloside treatment on day 2, more axons were seen caudal to the lesion site, and the lesion size was reduced.

Figure 4
Decrease in CSPG on BOTH acute and subacute treatment of xyloside

Figure 5
Xyloside treatment on day 2 increases IGF-1

Spatial organization of microglia/macrophages maintained following subacute xyloside treatment

Figure 6 Experiment
What type of phenotype do microglia/macrophages take on following CSPG activation?

- Cultured microglia on PDL or CSPG for 48 hours
  - Stained for IB-4, Hoechst (nuclei)
  - BrdU – proliferation
- PCR analysis for IGF-1 and MMP-2 and MMP-9

Microglia on CSPG have an ‘activated’ morphology and proliferate compared to those cultured on PDL
CSPG increased IGF-1 expression in microglia in vitro

MMPs, which are expressed by activated microglia and macrophages, were more abundant in CSPG cultures compared to PDL.

Why do CSPG degradation products promote recovery from spinal cord injury?

- Degradation of CSPG may form compounds with neuroprotective capabilities
  - chABC forms 6-sulfated disaccharide (CSPG-DS)
- Mice killed 14D after spinal cord injury
- Spinal cords were fixed, dehydrated, sliced, and stained for CS-56, BDNF, IGF-1

CSPG-DS – treated mice recovered better than PBS controls
CSPG levels unaffected

CSPG-DS treatment increased BDNF levels and IGF-1 levels
Increase in IGF-1 expression not limited to cells in margin of lesion
Acts through a different mechanism compared to CSPG

CSPG activate macrophages/microglia
  - Spatially
  - Increased in IGF-1 known for neuronal survival
  - Take on a beneficial, activated phenotype
Summary

- Potential feedback system in place for CSPG synthesis

Injured tissue → CSPG production → Activates beneficial macrophage phenotype

Macrophages express MMP2

Critique

- See expression of IGF-1 (microglial activation) and CS-56 (CSPG, glial scar formation) prior to spinal cord injury

- In vitro experiments cultured on PDL vs. CSPG
  - Should create more “glial-scar” like environments (CSPG-containing) to activate microglia or potentially astrocytes

- Beneficial to see microglial/macrophage organization using chimeric mice in CSPG-DS study
- Use of chABC (enzyme to degrade CSPGs)

Acknowledgements

- Dr. Shreiber
- Kate Fitzgerald
- Ileana Marrero-Berríos
- Biotechnology Training Program
Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury.

Lee H. McKeon R. and Bellamkonda R.
Ileana Marrero Berríos
Biomedical Engineering

Spinal cord injury (SCI) and glial scar

• After a SCI
  – A cascade of cellular and molecular responses culminates in the formation of a dense astroglial scar at the lesion site.
• The glial scar
  – Forms a barrier that blocks axon sprouting after SCI, reduces plasticity, inhibits healing process.
  – Is mainly composed of chondroitin sulfate proteoglycans (CSPGs) and reactive astrocytes.
• CSPGs
  – Class of axon growth inhibitors that are up-regulated SCI and contribute to regenerative failure.
  – Regulation peeks at 2-3 weeks after SCI.
  – Inhibition of synthesis by small interfering RNA (siRNA) or chABC digestion.

Chondroitinase ABC
• chABC
  – A bacterial enzyme isolated from Proteus vulgaris.
  – Digests the chondroitin sulfate glycosaminoglycans (CS-GAGs) of CSPGs.
  – Promotes axonal sprouting and functional recovery.
• chABC loses its enzymatic activity rapidly at 37 °C.
  – Use of repeated injections/local infusions.

Thermal stabilization of chABC using trehalose
• Trehalose is a natural sugar and a protein stabilizer.
  – Enzymes, such as RNAase and lysozyme have been stabilized with trehalose.
• Trehalose (20mM-1M) was coincubated with chABC:
  – Thermal stability analyzed by circular dichroism studies.
  – Enzymatic activity analyzed by SDS-PAGE (1, 2, 3 and 4 weeks).

Chondroitinase ABC enzymatic activity

Motivation
• There is a need to develop clinically viable methods for the spatially and temporally controlled delivery of chABC.
• Digest and maintain low levels of CSPGs after SCI.
  – Thermostabilize chABC using trehalose.
  – Develop a system for sustained local delivery of chABC in vivo.

Thermal denaturation curves of chABC using trehalose

Analysis of temperature-dependent conformation of chABC
• CD studies:
  – Midpoint transition temperature (Tm) increased by 8°C.

Normalized thermal denaturation curves of chABC, the dashed line represents chABC in 50 mM sodium phosphate buffer, and the solid line represents chABC in 1 M trehalose solution.


http://www.flickr.com/photos/43018525@N04/6822226418/
Enzymatic activity of TS-chABC

- The enzymatic activity of unstabilized and trehalose-stabilized chABC was evaluated by investigating the enzyme’s ability to digest the CSPG decorin.

![SDS-PAGE after 4 hour digestion](image)

- Controls: 1-intact decorin; 2-fresh chABC; 3-fresh penicillinase + decorin.

Microtube encapsulation of chABC

- Diacetylenic lipid microtubes form spontaneously while passing through a phase transition temperature in a controlled cooling process.

- After obtaining the lipid microtubes:
  - Added trehalose
  - Rehydrated with chABC or penicillinase.
- No protein exposure to heat or organic solvents.

Methods

- After obtaining the lipid microtubes:
  - Added trehalose
  - Rehydrated with chABC or penicillinase.

Encapsulated chABC enzymatic activity

- SDS-PAGE of encapsulated chABC enzymatic activity.

- Enzymatic activity of chABC and penicillinase released from microtubes up to two weeks.

Delivery of agarose-microtube-chABC scaffold after SCI

- Animal model: Male Sprague-Dawley rats
  - Dorsal-over-hemisection injury at T-10
  - Delivery system with agarose or single injection
  - Euthanized after 2 weeks
  - Immunohistochemistry analysis
    - 3B3-GAG stubs after digestion
    - CS-56: intact CSPGs

- Groups
  - Sustained delivery of TS-chABC (MTC)
  - Single injection conditions (SC, STC, STP)
  - Hydrogel-microtube scaffold (MTP, MT)
  - No injury, same procedure (Sham)
  - Injury and no treatment (injury/NoT)

Methods

- 3B3-GAG stubs after digestion
- CS-56: intact CSPGs

Immunohistologic analysis of CSPG digestion in vivo

- NoT: High CS-56, Low 3B3 (no digestion)
- MTC: Low CS-56, High 3B3 (digestion)

In vivo studies: hemisection SCI at T-10

- Schematic of spinal cord hemisection injury model and delivery of enzyme to the lesion site.
Sustained delivery of encapsulated chABC digests CSPGs effectively in vivo

Figure 2 cont.

Combination therapy of chABC and NT-3

- Animal model: Male Sprague-Dawley rats
  - Dorsal-over-hemisection injury at T-10
  - Delivery system or agarose gel. Single injection conditions were eliminated.
  - 6 weeks study with behavioral analysis.
    - Stride length
    - Immunohistological analysis: Cholera toxin B subunit (CTB)
      - Euthanized 3-4 days after CTB injection.

- Groups
  - Sustained delivery of TS-chABC / TS-chABC and NT-3 (MTC, MTCN)
  - Hydrogel-microtube scaffold (MTP, MTN)
  - No injury, same procedure (Sham)
  - Agarose gel with chABC and trehalose (GC)

Figure 3

Sustained delivery of chABC and NT-3 improves locomotor function

- Stride length was defined as a distance between consecutive steps with the same limb.

Figure S3

Sustained delivery of chABC and NT-3 promotes sprouting

(A) & (C) Microtube+trehalose+penicillinas

(B) & (D) Microtube+trehalose+chABC+NT-3

Conclusions

- Sustained local delivery of TS-chABC digests and maintains low levels of CSPGs after SCI.
- Combination therapy with chABC and NT-3 was found to enhance axonal sprouting and functional recovery after SCI.
- The hydrogel-microtube delivery system:
  - obviates the need for invasive, indwelling catheter/pump mediated delivery of chABC
  - enables combination therapy with neurotrophic factors
  - represents a promising approach to implementing chABC therapy after SCI.

Critiques: CatWalk study

- Stride length and gait analysis was performed with this test.
- No differentiation between blue and black bands.
- Abnormal paw print in MTP is not significantly different from Sham or MTCN groups.

CatWalk raw data in false color mode.

(A) Sham, (B) MTCN, and (C) MTP animals on the walkway at 6 weeks. All groups show a normal step sequence.
Acknowledgements

• Thank you!
  – Dr. Shreiber
  – Kate Fitzgerald
  – Kathryn Drzewiecki

• Biotechnology Training Program
A Review of Currently Marketed Antimicrobial Medical Devices and Emerging Strategies for Infection Prevention

Why should you be interested in device related infections?

Device Related Infections
What does it mean to be a “nidus” for infection?

What Forces are in Play in the Development of Infection Resistant Implants?

• Humanitarian
• Political
• Regulatory
• Legal
• Financial

Unsustainable Growth in Health Care Costs

According to the Congressional Budget Office (CBO), without any changes to federal law, total spending on health care will rise from 16 percent of the gross domestic product (GDP) in 2007 to 25 percent in 2025 and 49 percent in 2082, and net federal spending on Medicare and Medicaid will rise from four percent of the GDP to almost 20 percent over the same period.

The Cost of Negligence

• “Medical errors” cost between $17 billion and $29 billion per year
• Pre – 2009 most of the cost was shifted to Medicare and other outside payers

Source: http://www.nclsi.org

Sources:
1. Institute of Medicine (www.iom.edu)
Surgical site infection (infection in the chest) after surgery.

Focus on Infection

1. Object inadvertently left in after surgery
2. Air embolism
3. Blood incompatibility
4. Catheter associated urinary tract infection
5. Pressure ulcer (decubitus ulcer)
6. Vascular catheter associated infection
7. Surgical site infection-Mediastinitis (infection in the chest) after coronary artery bypass graft surgery
8. Certain types of falls and traumas
9. Surgical site infections following certain elective procedures, including certain orthopedic surgeries, and bariatric surgery for obesity
10. Certain manifestations of poor control of blood sugar levels
11. Deep vein thrombosis or pulmonary embolism following total knee replacement and hip replacement procedures

Source: CMS-1533-FC.

Catheter Associated Urinary Tract Infections (CAUTI)

CAUTIs are the most common type of healthcare-associated infection reported to the National Healthcare Safety Network (NHSN).
- Among UTIs acquired in the hospital, approximately 75% are associated with a urinary catheter
- Between 15-25% of hospitalized patients receive urinary catheters during their hospital stay

- 335 pages
- I have not read them...

Sources:
1) www.cdc.gov
2) www.ncsl.org
3) http://www.qualityforum.org
4) http://www.leapfroggroup.org/media/file/Leapfrog_Never_Events_Fact_Sheet.pdf
5) http://www.hopkinsmedicine.org/health/wellness-medicine/nursepractitioner/051130.html

The Joint Commission

R3 report: Issue 2, September 28, 2011
- Approximately 450,000 CAUTIs occur annually in hospitals
- The excess cost for nosocomial urinary tract infections (UTIs) ranges from $1,200 to more than $2,700 per case

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Level of Evidence</th>
</tr>
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<tbody>
<tr>
<td>...</td>
<td>...</td>
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</table>

Sources:
1. www.hopkinsmedicine.org/health/wellness-medicine/nursepractitioner/051130.html
2. www.cdc.gov
3. www.qualityforum.org
### CAUTI: Embedded Antimicrobials in use

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Structure</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrofurazone</td>
<td></td>
<td>The exact mechanism is unknown. Nitrofurazone inhibits several bacterial enzymes involved in the aerobic and anaerobic degradation of glucose and pyruvate.</td>
</tr>
<tr>
<td>Silver</td>
<td>Ag⁺⁺</td>
<td>DNA intercalation; protein inactivation</td>
</tr>
</tbody>
</table>

Nitrofurazone & Silver:
- Broad spectrum
- No common resistance mechanisms

### Antimicrobial Coated Catheters

**Strata NF (Rochester Medical)**

- FDA Approved in 1998
- Traditional 510k (K971627)
- Predicates:
  - C.R. Bard Bardex I.C. (K910318)
  - American Pharmacia Antimicrobial Foley (K871429)
  - Rochester Medical All Silicone Foley (K896053)
- Claim: "The catheter has been shown to provide a statistically significant reduction in the incidence of catheter acquired bacterial urinary tract infection during the first 5 days of infection."

Sources:
1. 5252_StrataNF_broch.pdf
2. K971627

### Antimicrobial Coated Catheters

**Johnson et al., Ann Intern Med. 17 January 2006;144(2):116-126**

Randomized or quasi-randomized clinical trials of antimicrobial urinary catheters currently marketed in the United States (i.e., nitrofurazone-coated and silver alloy-coated catheters) provide little or no data on their devices' effect on symptomatic urinary tract infection, morbidity, secondary bloodstream infection, mortality rates, or associated health care costs.

- Both nitrofurazone-coated and silver alloy-coated catheters seem to reduce the development of asymptomatic bacteriuria during short-term (<30 days) use, in comparison with latex or silicone control catheters.
- The magnitude of this effect varies among studies, seemingly in relation to the type of antimicrobial catheter (nitrofurazone or silver), patient sample (university or other), control group bacteriuria rate (high or low), and type of control catheter (latex or silicone).
- No trials directly compared nitrofurazone-coated and silver alloy-coated catheters.
- The clinical utility and cost-effectiveness of antimicrobial urinary catheters must be assessed in well-designed, adequately powered, randomized clinical trials.

### Antimicrobial Coated Catheters

**Hooton, et al., Clinical Infectious Diseases, 2010, Vol. 50, Issue 5, Pp. 625-663**

**Antimicrobial Coated Catheters**

In patients with short-term indwelling urethral catheterization, antimicrobial (silver alloy or antibiotic)-coated urinary catheters may be considered to reduce or delay the onset of CA-bacteriuria (B-I):

1. Data are insufficient to make a recommendation about whether use of such catheters reduces CA-UTI in patients with short-term indwelling urethral catheterization.
2. Data are insufficient to make a recommendation as to whether use of such catheters reduces CA-bacteriuria or CA-UTI in patients with long-term catheterization.

### Antimicrobial Coated Catheters

**Siddiqi et al., Nat Rev Urol. 2012 Apr 17(6):305-14:**

"Much recent research has focused on modification of the catheter surface by either coating or impregnation with antimicrobials or antiseptics. However, clinical trials that analyse cost-effectiveness and rates of antimicrobial resistance are awaited."

**StrataNF (nitrofurazone-coated) Product Brochure from Rochester Medical:**

"The most significant advancement in Foley catheter design in over 40 years"

Pre-market clinical trial involving 344 Patients:
- 90% reduction in bacterial CAUTI at 3 days.
- 83% reduction at 5 days for StrataNF when compared to a standard all-silicone Foley catheter.


### Cost Comparison

According to manufacturers’ list prices, the cost of an antimicrobial catheter is approximately 80 to 130% greater than for a non-coated catheter:

- $17.14 versus $7.43 for Bard silver-latex
- $12.80 versus $7.14 for Bard silver-silicone
- $10.00 versus $5.25 for Tyco silver-silicone
- $10.56 versus $4.56 for Rochester nitrofurazone - silicone

Sources:
1. Center for Reviews and Dissemination (http://www.crd.york.ac.uk)
2. www.catheterpros.com
Not just a Foley catheter problem...

Vascular Catheters ➔ Bacteremic catheter-related infection

- 200,000 cases per year
- 4.5 to 6.1 infections per 1000 catheter-days for medical/surgical intensive care units
- 20,000 deaths
- Cost for treatment ~$400 - $25,000

Source: http://www.ahrq.gov/clinic

Antimicrobial Venous Catheters

- 510k approved ~2000
- IFU: Antimicrobial treatment on the external surface of the catheter body as well as the entire fluid pathway of the catheter has been shown to be effective in reducing microbial colonization.
- Antimicrobial effectiveness was evaluated using in vitro methods, and no correlation between in vitro and clinical outcome has currently been ascertained. It is not intended to be used for the treatment of existing infections.

Source:
2) http://www accessingdata.fda.gov/cdrh_docs/pdf10/K100635.pdf

Teleflex hit with Class I recall of venous catheter

July 11, 2012

Teleflex Medical’s (STTX) Arrow International unit has been slapped with a Class I recall over its Arrowgard catheter, which the FDA says was dangerously mislabeled.

Designed to administer therapies within large veins, the catheter is labeled as containing no drugs, but in fact coated with chlorhexidine and silver sulfadiazine, the agency said. Patients who are allergic to these drugs could experience allergic reactions, including venal failure, possibly leading to death. The FDA tagged the recall with its most serious Class I designation.

Arrow launched a recall on May 3, sending a letter to its distributors asking them to quarantine the affected products, so they can be relabeled with accurate information. The FDA is asking patients already implanted with the catheter to report adverse events through its MedWatch program.

Source: http://www.flexomedicaldevices.com

Not just a catheter problem...

Sternotomy ➔ Mediastinitis

In 2008, 2.6% of sternotomies infected in Europe

Source: Santini, Abstract P1900 19th European Congress of Clinical Microbiology and Infectious Diseases

The multi-centre US National Healthcare Safety Network (NHSN) reported in 2008 their 2-year data (2006-’07) for CABG SSI rates of 3.29% (2,259/68,647)


Gentamicin Loaded Collagen Sponge

Innocoll

- CollaRx® Gentamicin Surgical Implant (excluding the US) is a perioperative surgical implant comprised of a lyophilized collagen matrix impregnated with the broad spectrum antibiotic, gentamicin (as the sulfate salt).
- Large European multicenter trial demonstrated reduction in infection: 4.0% (11/272) in the gentamicin group and 5.9% (16/270) in the control group
- CE Mark Approved
- Granted Fast Track designation by FDA in 2008 – Moved directly to Phase III Trial

Sources:
1) www.innocoll.com
2) www.medgadget.com

CollaRX Clinical Studies

Results: In the primary analysis, there was no significant difference in sternal wound infection:
63 of 753 patients randomized to the gentamicin-collagen sponge group (8.4%) compared with 65 of 749 patients randomized to the control group (8.7%) (P=.83)

Conclusion: Among US patients with diabetes, high body mass index, or both undergoing cardiac surgery, the use of 2 gentamicin-collagen sponges compared with no intervention did not reduce the 90-day sternal wound infection rate.
**CollaRX Clinical Studies**

**Gentamicin-collagen sponge reduces sternal wound complications after heart surgery: A controlled, prospectively randomized, double-blind study**

**Material and Methods:**
- Patients: 51 patients
- Control group: 25
- Experimental group: 26
- Kebony: 1.5 mm X 1.5 mm X 5 mm
- Intravenous gentamicin antibiotic therapy: did not differ
- Sternal closure and the surgical incision
- Cultures: took cultures of the entire sternum over the dressing
- Results: no infection
- Follow-up: 6 months
- Conclusion: prevented sternal wound infections

**References:**
- Slide borrowed from Tom Bauer, M.D. (with permission)

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**Not just a sternum problem...**

- **Rate of infection with closed fracture:** 1.5%, Open fracture 3-40%
- **60% of open fractures are contaminated at injury**
- **Over 30% of US pop is colonized by S aureus, and 4% of Healthcare is MRSA colonized**
- **Staph in Nares 2-9x increase in SSI, with 80-85% of isolates matching**

**Sources:**
- Harris et al., Injury 2006
- Evans, et al., JBJS 2009

---

**Pathologist’s Perspective: Periprosthetic Infections are bad**

- The cost to treat periprosthetic infections is substantial
- And is even higher for MRSA. The mean cost of treating MRSA infection was $107,264.00 compared to $68,053 for infections with sensitive organisms*


**Slide borrowed from Tom Bauer, M.D. (with permission)**

---

**Orthopaedic device infections may be more common than we think!**

**Literature review reveals high infection rates:**
- 1% for instrumented spine fusion
- 2% for joint prostheses
- 5% for fracture fixation devices

**Sources:**
- 1. Backy et al., Int Orthop. 2011
- 3. Darouiche et al., Clin Infect Dis 2001
- 4. Widmer et al., Clin Infect Dis 2001
- 5. Boxma et al., Lancet 1996
- 6. Zimmerli et al., JAMA 1998

**Since complications from device infection are devastating, even low-end estimates of infection rate are cause for great concern. However, it was recently brought to light that the infection problem in Orthopaedics could be understated.**

---

**What’s on our explants? Studies of “aseptic” explants...**

**Moojen et al., 2010: Used broad range polymerase chain reaction with novel diagnostics to determine infection rate in 176 explanted hips**
- Up to 13% of patients diagnosed with aseptic loosening were infected

**Turney et al, 1999: Used sonication followed by cultures and PCR to determine the infection rate of 120 explanted hips**
- 72% of devices showed evidence of bacteria as measured by PCR
- 22% infection rate when using strict anaerobic culturing technique
- Turney et al., states: “Our results indicate that the incidence of prosthetic joint infection is grossly underestimated by current culture detection methods.”

---

**Increased Yield of Cultures After Sonication**

**Routine specimen**

**After sonication and centrifugation**

**Slide borrowed from Tom Bauer, M.D. (with permission)**
Orthopaedic Antimicrobial Devices

Antimicrobial Bone Cements
PMMA + Antimicrobial

Others:
Smartset GHV - Gentamicin
Versabond AB - Gentamicin

Gentamicin
Tobramycin

IFU: The cement is indicated for use in the second stage of a two stage revision for total joint arthroplasty after the initial infection has been cleared.

Antimicrobial Cement Impact/Cost Analysis
Cummins et al., JBIJS, 2009
- Calculated impact of antimicrobial bone cements in “Quality Adjusted Life Years” (QALY)
- Found device improve patient outcomes
- Calculated that gain is worth no more than $650 per case
- Additional cost for antibiotic bone cement: $600

Antibiotic Coated Metallics
UTN PROtect
- Polylactide coating (50 microns)
- 10-50 mg gentamicin per nail
- CE Marked 2006
- Based on studies of release kinetics of the UTN PROtect implant (diameter 8 mm, length 330 mm) in deionised water, over 40% of the antibiotic is released within 1 h, 70% within 24 h and 80% within 48 h after implantation

Gentamicin Eluting Nail – Clinical Trial
Non-randomized study: Patients all received coated nails
No infections (0/19 infected)
No signs of adverse effects attributable to coating
Total Enrollment: 100
Estimated completion Date: September 2013
Sources:
1) http://clinicaltrials.gov
2) Fuchs et al., 2011

Antimicrobial Bone Void Filler
CE Mark Approval (Not cleared by FDA)
Contains 4% Tobramycin Sulfate

IFU:
OSTEOSET™ T Bone Graft Substitute is intended to be gently packed into non-load bearing voids in long bones. These bone voids may be surgically created osseous defects, osseous defects created from traumatic injury to the bone or osteomyelitis. The pellets provide a bone void filler that resorbs and is replaced with bone during the healing process.

Not Just an Orthopaedic Problem...
Infection in Cardiac Electrophysiology

<table>
<thead>
<tr>
<th>Years</th>
<th>Pacemakers</th>
<th>ICDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996 (%)</td>
<td>2.73</td>
<td>1.63</td>
</tr>
<tr>
<td>2003 (%)</td>
<td>5.82</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Source: www.theheart.org

The consequences of device infection:
- Average treatment cost of $146,000 per case
- Mortality rate for endocarditis: ~30%
Colonization of Pacemaker Can and Leads

Infection Routes:
1) Nosocomial – Pre-implantation
2) Nosocomial – SubQ Pouch contamination
3) Nosocomial – Lead Insertion
4) Hematogenous – Colonization of Can or Leads

Antimicrobial Devices for CRM

- Polypropylene sleeve coated with bioabsorbable polymer which elutes minocycline and rifampin (7-9 days)
- Approved by FDA on the basis of COMMAND trial

TYRX purchased by Medtronic for 160M + royalties

Summary of Currently Marketed Antimicrobial Medical Devices

- They all work by eluting antimicrobials to create a bactericidal zone

Good Review Article

Other Approaches to Create Infection Resistant Implants?

1. Design Surfaces which resist bacterial attachment
2. Tether antimicrobials to surface to kill bacteria as they come into contact with an implant

Bacterial Attachment and Biofilm Formation
Deficit Reduction Act of 2005

Deficit Reduction Act Sec. 5001. Hospital Quality Improvement: (c) Quality Adjustment in DRG Payments for Certain Hospital Acquired Infections (1) Amends Section 1886(d)(4) of the Social Security Act by adding language that states that for discharges occurring after October 1, 2008, the diagnosis related group (DRG) assigned may not result in a higher payment based on a secondary diagnosis associated with conditions identified by the secretary that could have reasonably been avoided through the application of evidence-based guidelines. Hospitals will be required to report the secondary diagnosis present on admission of the patient.

Specific criteria for selection of the conditions were provided as follows:
1. The condition must be associated with a high cost of treatment or high occurrence rates within hospital settings.
2. The condition results in higher payment to the facility when submitted as a secondary diagnosis.
3. The condition can reasonably be prevented by adoption and implementation of evidence-based guidelines.

Source: Pub. L. 109-171 (DRA)

Deficit Reduction Act of 2005

Obamacare
Jul 5, 2012 in Genetic Engineering & Biotechnology News written by Alex Philippidis:

R&D Changes Foreseen After Supreme Court Obamacare Decision
“innovative drugs that offer clear superiority over existing products likely among beneficiaries of overhaul.”

• Companies will continue to focus R&D on what will be reimbursed
• Incremental improvements may not be moved forward
• Obama agency has suggested that cutting exclusivity period to 7 years could save $4 billion over ten years
• Effect on Medical Devices?

Source: http://www.genengnews.com
Overview

- Bactericidal surfaces
- Goal
- Methods/Results
- Summary of Findings
- Strengths and Future Work

Bactericidal Surfaces

- Chemically-modified surfaces
  - Polyethylene glycol (PEG)-surface.
    - Repels microorganism, does not kill
  - Release-based materials.
    - Impregnation with antimicrobial agents

Shortfalls
- Need a liquid medium, not effective against airborne bacteria
- Antibacterial activity fades away with time
- Bacteria becomes resistant

Previous Work

- N-alkyl-PEI functionalized surfaces resulted in a microbicidal surface against airborne, waterborne bacteria, and fungi
- N-alkyl-PEI surface kills bacteria upon direct contact

Goal

To determine the practical utility of the bactericidal effect of N-alkyl-PEI-functionalized surfaces

N-alkyl-PEI Surface Preparation

1. Aminopropyltriethoxysilane-modified glass slide
2. Polyethylenimine (PEI)
3. N-alkylation produces quaternary compound (net positive charge)
1. To investigate the time scale of microbial action of immobilized N-Alkyl-PEI.

**Method**

- **Live/Dead Assay:**
  - Membrane-permeable green fluorescent nucleic acid stain → SYTO-9
  - Not membrane-permeable red fluorescent nucleic acid stain → Propidium iodide (PI)
- Bacteria solution fluorescent incubated on N-alkyl-PEI derivatized glass slide and amino-glass slide (control)
- Analysis by fluorescence microscopy at 20 and 120 minutes
- Emulates real-life conditions (cells grown at 37°C)

**Results**

- A and B show intactness of bacteria membranes on untreated glass slide
- C: At 20 minutes majority of bacterial cells show compromised membrane (red)
- D: After 2 hours virtually all cells look red

2. To validate efficiency of bacterial inactivation

**Method**

- Each measurement done in triplicate
- Percentages of live/dead bacteria obtained by counting at least 200 cells.
- Test was done in surface-bound and floating bacteria
- Initial bacterial count 2.4x10^6 cfu/ml

**Results**

- N-alkyl-PEI incubated with 40ml of E.coli in a range of concentrations: 2 x 10^8, 6x10^7, 2x10^8, 1.2x10^8, 1.8x10^8 cfu/ml
- Viable bacterial measured at different times from 20 min-120 min

**Table 1:** Bactericidal action of N-alkyl-PEI functionalized slide toward treatment E.coli

<table>
<thead>
<tr>
<th>Time of exposure (min)</th>
<th>Control</th>
<th>Membrane-permeable</th>
<th>Not membrane-permeable</th>
<th>On slide (cfu)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
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<td>10</td>
<td>2.8 x 10^6</td>
<td>1.2 x 10^6</td>
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</table>

**Results**

- All bacteria are killed
- d.e. Calculations show that 4 of bacteria killed is 2.2 x 10^9
- Approx 1 cm^2 of slide is able to kill 0.8 x 10^8 cells
- Approximating cell size shows that all cells adhere to surface die
- d.e. Bactericidal action is absent beyond first layer of adsorbed bacteria
4. To quantify time-dependent measurements of bacterial inactivation

**Method**
- N-alkyl-PEI incubated with 2ul of 1x10^9 cells/ml over 1cm².
- Viable bacterial measured during 6 hours

**Results**
- There is no detachment of bacteria once they are adsorbed
- Control (b-curves) shows no significant loss of viability
- Derivatized surfaces on both types of bacteria (a-curves) show no viable bacteria even after 6 hours.

5. To evaluate development of resistance in airborne bacteria

**Method**
- 11 sequential biocidal assays independently
- Bacterial populations for each assay originated from a single colony of the preceding assay
- Percentage of inactivation calculated as a linear function y=ax+b
- Measurements were done in duplicates

**Results**
- Bactericidal efficiency remained unchanged
- Calculated slope is statistically insignificant, show no change
- Neither type of bacteria become resistant to N-alkyl-PEI

6. To evaluate the effect of antimicrobial surfaces on Mammalian cells

**Method**
- COS-7 cells - monkey kidney cells
- Live/dead assay
  - Healthy cells: produce red fluorescent C12-resourfin and avoid SYTOX green fluorescent uptake
  - Damaged membrane cells: unable to produce C12-resourfin and accumulate SYTOX green
- Kidney cells are incubated with a derivatized glass+fluorescent probes
- Fluorescence was monitored for 2 hours

**Results**
- 628 of 664 cells in the field of vision (95%) remained viable

Summary of Findings
- N-alkyl-PEI-derivatized antimicrobial acts as a non-selective permeabilizer of bacterial membrane causing their fatal damage.
- PEI chains must be sufficiently long to reach bacteria
- The magnitude of the bactericidal action is determined by surface area available for direct contact with bacteria
- Bacteria does not develop resistance to the bactericidal action
- Antimicrobial activity can be regenerated by washing the surface
- N-alkyl-PEI causes rapid and efficient bacterial inactivation
- N-alkyl-PEI is deadly to bacteria but has no detrimental effect on Mammalian cells

Strengths

- Addresses current shortfalls
  - Airborne bacteria
  - Resistance
- Shows initial studies of biocompatibility with Mammalian cells studies

Future Applications

- Washable surface with fast bactericidal action optimal for surgical tools
- Suitable for short-term medical devices
  - Catheters
  - Drainage tubes
Questions?
Anti-Fouling Chemistry of Chiral Monolayers: Enhancing Biofilm Resistance on Racemic Surface

Debjyoti Bandopadhyay, Deepali Prashar, Yan-Yeung Luk

Presentation by: William Pfaff

Self-Assembled Monolayers (SAMs)
- Platform technology used to investigate how cells and biological molecules interact with material surfaces
- Alkanethioles spontaneously and irreversibly assemble on a gold substrate
- Functional groups can be attached to the alkanethiol tails to present a molecularly uniform surface

Antibacterial Polyol Surfaces
- Various SAM functional groups tested to determine which surface chemistry is the most "bio-inert"
- Polyol functional groups resist protein adsorption
- Poly-ethylene glycol (PEG) discovered to resist bacteria
- Luk, Mrksich and Whitesides discover certain polyol functional groups resist protein adsorption better than PEG

Experiment Goals
- Knowing that polyol terminating SAMs resist protein adsorption and mammalian cell adhesion, this paper sets out to test:
  - Will polyol-terminating SAMs resist bacterial adhesion? Protein adsorption?
  - Does the chirality of the polyol functional group affect bacterial adhesion? Protein adsorption?

Chiral Alditols and Aldonamides
- Chirality is the asymmetrical property of similar 3-dimensional molecules
- Chirality may affect how cells interact with surface molecules
- A pure chirality vs a racemic mixture may result in different surface properties
- Enantiomers of gulitol and mannonamide used for this study

SAM Micro-Patterned Surfaces
- Microcontact printing used to create 135 um wide circular patches of pentadecanethiolis (methyl terminus)
- Surrounding SAM surface composed of either D, L, or a racemic mixture of polyol-terminating alkanethiols
- One group is gulitol-terminating, the other is mannonamide-terminating
**Cell Culture Procedure**

- SAM modified gold substrates placed in a dual channel flow cell incubated with broth containing E. coli for 1 hour
- Culture media then continuously flown through channels

**Staining Fluorescence**

- E. coli cells engineered to express red fluorescent protein
- Fluorescent images captured every day using a high resolution microscopy camera
  
  After 6 hours:
  
  A) Fluorescent bacteria cultured on pentadecanethiol islands surrounded by exposed gold surface
  
  B) Fluorescent bacteria cultured on pentadecanethiol islands surrounded by D-gulitol
  
  After 3 days:
  
  C) Fluorescent bacteria concentrations reverse on pentadecanethiol islands surrounded by D-gulitol

**Surface Plasmon Resonance (SPR)**

- Technique used to measure adsorption of material onto a planar metal
- 4 different SAMs were placed in flow cells, with a medium containing bovine serum albumin proteins flowing across for a brief period
- Intensity of SPR recorded on surface of SAM over time

**Summary of Findings**

- Initially E. coli adhered to the hydrophilic polyol SAMs and not to the hydrophobic methyl SAM within first 6 hours
- Over the course of 3 weeks E. coli forms a biofilm colony on the hydrophobic methyl SAM and detached from the polyol surface
- Racemic mixture of gulitol enantiomers resist bacterial cell adhesion better than pure chiral SAMs
Future Applications

Polyol coated surfaces may be developed for implantable medical devices which require long-term resistance to biofouling

Pros:
- Long term resistance to protein attachment and biofouling from bacteria
- Biocompatible and bio-inert

Cons:
- No mammalian cell attachment - not good for load-bearing implants or scaffolds for tissue engineering
- Does not prevent bacterial attachment prior to implantation

Future Work

Why does chirality affect the rate of biofilm formation?
- AFM and STM to characterize surface properties - does chirality cause imperfections on SAM surfaces that allow proteins to adhere?
- How can we quantify bacterial attachment better?
- Use QCM to profile cell attachment/detachment rates?

Bactericidal vs Protein Resistant

Bactericidal surfaces:
- Disinfects what it touches in a short amount of time
- Effective against planktonic and airborne bacteria
- Ideal for hospital instrumentation and short-term medical implants
- Bacteria ruptures, proteins coat the surface and is no longer bactericidal

Protein resistant surfaces:
- Resists biofilm formation over a long period of time (weeks)
- Ideal for long-term removable implants
- Bacteria (reversibly) attaches to surface
- Mammalian cells do not adhere (material cannot interact with or bear loads with human tissue)
- Only works in water

References

Michal Ejgenberg and Yitzhak Mastai (2012). Crystallization on Self Assembled Monolayers, Advances in Crystallization Processes, Dr. Yitzhak Mastai (Ed.).

Thank you to
Jordan Katz
Nir Nativ
Nathalia Garcia
and everyone in the Biotechnology Training Program!
Outline of today’s talk

- Overview - mass spectrometry in proteomics
- How mass spectrometers work
- Looking at spectra - mass and isotopes
- Quantitative proteomics

What does a mass spectrometer do?

- Measures mass/charge (m/z) of intact ions and fragments

Use of mass spectrometry in proteomics research

protein identification
protein processing: proteolytic post-translational modifications (100’s)
quaternary structure complex formation steady-state levels turnover rates structural studies (H/D exchange)
comparison of samples (disease & controls)

Mass spectrometry is an enabling technology*

in the field of proteomics

<table>
<thead>
<tr>
<th>Peptide Sequencing</th>
<th>Edman degradation</th>
<th>Mass spectrometry</th>
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<tbody>
<tr>
<td>Material required</td>
<td>~pmol (6 x 10^11 molecules)</td>
<td>xfmol 6 x 10^6 molecules</td>
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<td>Time (data collection)</td>
<td>~10 hours</td>
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*An enabling technology is an invention or innovation, that can be applied to drive radical change in the capabilities of a user or culture. Enabling technologies are characterized by rapid development of subsequent derivative technologies, often in diverse fields. Equipment and/or methodology that, alone or in combination with associated technologies, provides the means to increase performance and capabilities of the user, product or process.

The mass of a single peptide provides sequence constraints but is not sufficient for identification

The precursor mass is not enough information for ID

Wilhelm Haas
Collision-Induced Dissociation (CID)

- Kinetic energy of parent ions is increased
- Parent ions undergo energy converting collisions
- Parent ions fall apart into product ions and neutrals
- Also referred to collision-activated dissociation (CAD)

Typical CID fragmentation pattern of peptides

Combined residue mass for two amino acids

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<th>Amino Acid</th>
<th>Combined Mass</th>
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</table>

Sequencing a peptide ion using tandem MS (MS/MS)

1. MS
2. Peptide ions are measured in MS/MS
3. Collide with inert gas (CID) to fragment
4. Fragment ions are measured in MS/MS

A typical proteomics experiment

- Separate microgram quantities of peptides on a capillary C18 column
- We can identify 10,000 peptides in a 90 min run

Wilhelm Haas
How do mass spectrometers work?

Mass analyzers use forces to manipulate ions

\[
\bar{F} = ma = m \frac{d\bar{v}}{dt} = z(E + \bar{v} \times \bar{B})
\]

\[
\frac{m}{z} \frac{d\bar{v}}{dt} = E + \bar{v} \times \bar{B}
\]

Time-of-flight (TOF) mass analyzer

- Ions are formed in pulses.
- The drift region is field free.
- Measures the time for ions to reach the detector.
- Small ions reach the detector before large ones.

\[
\text{Energy uptake is } E_{\text{u}} = qV = ezV \text{ where } e = \text{charge/electron}
\]

\[
\text{Conversion of potential energy to kinetic energy. } ezV = \frac{1}{2} mv^2
\]

\[
\text{The drift region is field free. Known distance, } d.
\]

\[
\text{Measures the time for ions to reach the detector. } v = \frac{d}{t}
\]

\[
m/z = \frac{2eV^2}{v^2} = \frac{2eVd^2}{t^2}
\]

Quadrupole mass analyzer

- Oscillating electric fields, operates as a mass filter.
- Has four parallel metal rods.
- Lets one mass pass through at a time.
- Can scan through all masses or sit at one fixed mass.

Quadrupoles have variable ion transmission modes

- Mass scanning mode (let different m/z through as function of time, collect mass spectrum)
- Single mass transmission mode (let single m/z through, measure intensity)
Triple quadrupole MS (tandem in space)

How do mass spectrometers work?

Matrix Assisted Laser Desorption/Ionization (MALDI)

1. Sample is mixed with matrix (X) and dried on plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules (M) are ionized by proton transfer:
   \[ XH^+ + M \rightarrow MH^+ + X \]

**Sample plate**

**Laser**

**MH^+**

Vacuum

Grid (0 V)

\( \pm 20 \text{ kV} \)

MALDI

Matrix Assisted Laser Desorption/Ionization

Electrospray Ionization (ESI)

- Frequently used with liquid chromatography (LC-MS)

- Nanospray (typically \( \sim 0.2 \mu \text{L/min} \)) do not use sheath gas.

Electrospray Ionization (ESI)

- Frequently used with liquid chromatography (LC-MS)

- \( \mu \text{LC} \) (M+H)^+

Protonated Molecules

Taylor Cone

Plume

Nanoparticle

To MS

Mass spectrometers are frequently named based on their ion source & mass analyzer

Electrospray Ionization (ESI)

- Frequently used with liquid chromatography (LC-MS)

- Nanospray (typically \( \sim 0.2 \mu \text{L/min} \)) do not use sheath gas.
Mass Spectra

• Mass units
• Isotopes
  – the good, the bad & the ugly

How is mass defined?

• Numerical value to the intrinsic property of “mass” is based in reference to the most abundant isotope of carbon, \(^{12}\text{C}\) (6 protons & 6 neutrons).
• One unit of mass is defined as a Dalton (Da).
• A Da is defined as 1/12 the mass of one \(^{12}\text{C}\) atom.
• Thus, one \(^{12}\text{C}\) atom has a mass of 12.0000... Da.

Most elements have >1 stable isotope

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.11% of C atoms have an extra neutron, making their mass 13 Da.

Isotope composition of molecules depends on molecular formula and isotope distribution of component atoms (binomial distribution).

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotope</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>1H</td>
<td>99.985</td>
</tr>
<tr>
<td></td>
<td>2H</td>
<td>0.015</td>
</tr>
<tr>
<td>Carbon</td>
<td>12C</td>
<td>98.890</td>
</tr>
<tr>
<td></td>
<td>13C</td>
<td>1.110</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>14N</td>
<td>99.630</td>
</tr>
<tr>
<td></td>
<td>15N</td>
<td>0.370</td>
</tr>
<tr>
<td>Oxygen</td>
<td>16O</td>
<td>99.759</td>
</tr>
<tr>
<td></td>
<td>17O</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>18O</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Monoisotopic peak, \(^{12}\text{C}_{\text{1}}\) ~ 99% of total (0.989)\(^{1}\)

Isotope pattern (\(\text{C}_{1}\))\(^{+1}\)

Monoisotopic peak, \(^{12}\text{C}_{60}\) ~ 51% of total (0.989)\(^{60}\)

Isotope Pattern (\(\text{C}_{60}\))\(^{+1}\)

Monoisotopic peak, \(^{12}\text{C}_{300}\) ~ 4% of total (0.989)\(^{300}\)

Isotope Pattern (\(\text{C}_{300}\))\(^{+1}\)
Effect of isotope abundance on mass measurements

Isotope clusters allow charge state determination

Resolution

\[ R = \frac{M}{\Delta M} \]

Resolution = minimum peak separation, \( \Delta M \), which allows to distinguish two ion species

\( \Delta M = \) full width at half maximum (FWHM)

Resolution = \( M/\Delta M = 500/0.5 = 1000 \)

Monoisotopic mass

When the isotopes are clearly resolved the \textit{monoisotopic mass} is used as it is the most accurate measurement.

Spacing of adjacent isotope peaks (\( \Delta m/z \), measured) gives \( z \) as known \( \Delta m \)

Average mass

Average mass corresponds to the centroid of the unresolved peak cluster

When the isotopes are not resolved, the centroid of the envelope corresponds to the weighted average of all the isotope peaks in the cluster, which is the same as the average or chemical mass.

Why we typically analyze peptides instead of intact proteins

- Protein heterogeneity:
  proteolytic processing
  post translational modifications
- Stability in gas phase
- Natural isotope abundance
- Better resolution and accuracy at lower masses
Quantitative proteomics

- Label-free
- Stable isotope labeled ($^{13}$C, $^{15}$N, and/or $^{18}$O)
  - Metabolic labeling
  - Synthetic peptides and proteins
  - Chemical modification

Quantitative analysis using an internal standard

Parting message

Progress in science depends on
new techniques,
new discoveries, and
new ideas,
probably in that order

Sydney Brenner, 20 March 1980
_Biology in the 1980's_, talk at the
Friedrich Miescher Institute, Basel, Switzerland
MS3 eliminates ratio distortion in isobaric labeling-based multiplexed quantitative proteomics

Lily Ting, Ramin Rad, Steven P. Gygi, and Wilhelm Haas

1Department of Cell Biology, Harvard Medical School, 240 Longwood Ave, Boston Ma, 02115, USA


Isobaric Labeling Mass Spectrometry

• Isotope labeling mass spectrometry strategies in proteomics
  – Provide quantitative comparisons in multiplexed manner
  – Allow for up to 10 samples analyzed at one time
• Several Commercial Reagents
  – Isobaric tags for Relative and Absolute Quantification (iTRAQ)
    – iTRAQ 4plex
    – iTRAQ 8plex
  – Tandem Mass Tags (TMTs)
    – TMT 6plex
    – TMT 10plex


Tandem Mass Spectrometry (MS2 or MS/MS)

• Proteins are isolated from cell lysate
• Digested enzymatically
• Peptides are separated using liquid chromatography
• MS1 of eluting peptides is taken
• Computer makes prioritized list of peptides for further fragmentation
• MS2 ensues on each targeted peptide
• Compare results to database for protein identification


iTRAQ 4plex Tagging Chemistry

• Peptide-reactive group forms amide linkage to any peptide amine
  – Placed at N termini and lysine side chains
• Reporter groups have masses (114, 115, 116, and 117)
• Mass balance groups are neutral with varied mass
  – Achieved using asymmetric incorporation of heavy isotopes
• Following amide bond fragmentation, mass balance moiety is lost


Relative Quantification

• Relative areas of peaks of MS2 reporter ions m/z correspond with the proportions of the labeled peptides (ii)

Absolute Quantification

- Can add a known amount of a tagged peptide to act as an internal standard


MS2 Quantification “Interference Effect”

- Accuracy and precision of quantitative data suffer due to contaminating ions co-isolated and co-fragmented with target ions...
  - Can lead to underestimation of protein expression changes

Objective

- A multi-proteome model sample was used to accurately measure the extent of the interference effect on quantitative data from a large scale proteomics experiment.
  - This model was used to evaluate several analytical strategies as potential solutions and show that collecting an additional isolation and fragmentation event (MS3 scan) eliminates the interference effect.

Methods-Model of Interference Effect

S. cerevisiae (yeast) Human HeLa Cells

LC-MS2

Separates by hydrophobicity

TMT 6plex

Strong Cation Exchange Chromatography (SCX)

Separates by charge density

Expected Results

- Without interference, expect same ratios as samples
- With interference, ratios are underestimated

Results-Model of Interference Effect

- Human peptides interference leveled out yeast reporter ion intensities
- Yeast peptides interference can be seen in tags 129,130, and 131 of human peptides
Loss of accuracy was accompanied by loss of precision. A 60% increase in standard deviation was observed due to interference.

Yeast without interference: 131:130, 130:129, 131:129
Yeast with interference: 126:127, 127:128, 126:128

Four Proposed Analytical Strategies to eliminate the interference effect:
1. Estimation of interference based on full-MS data
2. Sample Fractionation
3. Reduction of the Precursor Ion Isolation Width
4. MS3 data acquisition

Estimation of interference based on full-MS data:
- Estimated the number and intensity of contaminating peptide ions by isolation specificity (IS)
  - Relative fraction of target ion intensity compared to the complete ion intensity within the precursor ion isolation window
  - A measure of purity
- Compared median reporter ion ratios for peptide ions with IS > 0.9 and IS < 0.8 but only a modest reduction of interference was observed

Sample Fractionation:
- Unfractionated cell lysate was compared to lysate fractionated by SCX
- Resulted in a lower reduction of interference relative to isolation specificity method

Reduction of the Precursor Ion Isolation Width:
- Precursor ion isolation window was narrowed from 2 to 0.5 m/z
- Reduced interference more than other two methods
- 22% reduction of unique peptide identifications

MS3 data acquisition:
- MS1 scan identifies target peptides for MS2 scan
- MS2 isolates identified ion and fragments
  - Each precursor ion has its own MS2 scan
  - Reporter tag is not fragmented
- MS3 fragments the most abundant peptide from MS2 scan
  - Removes reporter tag
  - Quantification
Disadvantages
• 12% decrease of unique proteins identified
• Extra step results in reduced acquisition speed
  – About 10% reduction of duty cycle
• Authors believe the quality of the quantitative data provided by the MS3 method justifies the loss in analytical depth

Conclusions
• A multi-proteome sample strategy was presented to accurately evaluate the extent of interference when using isobaric labeling in quantitative mass spectrometry-based proteomics
• MS3 method provides an experimental solution to remove this interference and eliminate the ratio distortion problem

Acknowledgements
• Dr. Peter Lobel
• Andrea Gray
• Antoinette Nelson
• Biotechnology Training Program
References

Nanoscale Approaches for HIV Eradication and Prevention

Antoinette Nelson
Advisor: Dr. Patrick J. Sinko
Fall 2013

New HIV infections = ~ 2.7 million [2.4 – 3.0 million]
Deaths due to AIDS = ~ 2.0 million [1.7 – 2.4 million]

HIV Virus

• T-cells
• Monocytes
• Macrophages
• Dendritic Cells
• Microglial cells

Types of Anti-HIV Therapeutics
• Nucleoside reverse transcriptase inhibitors
• Non-nucleoside reverse transcriptase inhibitors
• Protease inhibitors
• Fusion inhibitors
• HAART (Highly Active Antiretroviral Therapy)

Obstacles for HIV Therapy

• Macrophages, T-cells and dendritic cells occupy areas difficult to penetrate with drugs
• Occupy the brain, lung, lymph node, spleen, bone marrow, GI tract
• Macrophages more resistant to the cytopathic effects of the virus
• Cellular reservoir for long term persistence of HIV-1
• Produces and harbors the virus for a longer period
• HAART efficiency limitation

98% of lymphocytes are distributed among the lymphoid tissues

• GALT- gut associated lymphoid tissues
• Protect body from invasion
• Digestive system is an important component of immune system
• Lymphoid tissues heavily infected upon initial infection
• Sustained drug release needed
Challenge
To eliminate residual reservoirs of HIV that persist in individuals despite chronic antiretroviral therapy

Project Goal
To develop nanocarrier-based approaches to eliminate active cellular reservoirs of HIV in the gut and mesenteric lymph nodes by delivering antiretroviral agents locally in effective and sustained concentrations to inhibit HIV transmission by blocking viral entry and disrupting viral synapses

NP Formation
PS-PEG Block Copolymer
Hydrophilic Block
Drug
Imaging Agent
Hydrophobic Block
Organic Solvent
Aqueous Solvent
Nanoparticle

Flash Nanoprecipitation
Organic Stream
Water
Nanoparticles
Mixing Chamber
~100 μl

Foams to Administer NPs
continuous phase; V_{p,c}
gas phase; V_{p,g}

Distribution of NPs in Foam

Non-Alcoholic Foam at Room Temperature
30 sec
5 min
15 min
30 min
1 h
2 h
Optical and MRI Images of Foam Distribution in Colon

Project Goals

1. Optimize surface ligands
2. Systemically vary components
3. Perform in vitro binding and transport tests
4. Formulate and assess foam distribution and residue

Acknowledgements

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- Dr. Patrick J. Sinko
- Dan Myers
- Mahta Samidezah
- Zoltan Szekely, PhD
- Steven Johnson
- Xiaoping Zhang, PhD
- Derek Adler
CRISPR – A Precise Method To Engineer Genomes

Dr Samuel F Bunting
Department of Molecular Biology and Biochemistry
Biotech Training Program, Rutgers University
March 7, 2014

CRISPR: What Is It And Who Cares?

DNA

NUCLEASE

GUIDE RNA

The ability to efficiently cut any piece of DNA, inside living cells, provides unprecedented power to manipulate living organisms, manage disease states, and conduct research.

Components of the RNA-Guided Nuclease
1. The Nuclease – Cas9

HHN domain
RuvC domain
Cutting domains of the ‘double nickase’

Structure of S. pyogenes Cas9

Components of the RNA-Guided Nuclease
1. The Nuclease- The HHN domain

The HHN domain is found among a class of homing endonucleases.

Characteristics of homing endonucleases
- Long consensus sequences (30 nucleotides)
- Tolerate mismatches in consensus sequence
- Found in archaea, prokaryotes, eukaryotes
- Act as monomers, dimers or RNA-protein complexes

Characteristics of “HHN” homing endonucleases
- Pairs of Histidine-Asparagine-Histidine (HHN) act as zinc fingers to bind DNA elements
- Also found in ‘colicin’ nucleases, such as E9 Dnase.

Components of the RNA-Guided Nuclease
1. The Nuclease- The RuvC domain

- The RuvC domain has homology to the E. coli Holliday Junction Resolvase
- Homology to Retroviral Integrase, RNaseH

Claw-like structure
RuvC domain
Components of the RNA-Guided Nuclease
2. The Guide RNA

(Nishimasu H, et al, Cell, Feb 27, 2014)

Action of the ‘Double-Nickase’

RNA Hairpins in pre-miRNA molecules
Evolution of CRISPR
CRISPR = Clustered Regularly Spaced Interspaced Short Palindromic Repeats

“direct repeats, varying in size from 21 to 37 bp, interspaced by similarly sized non-repetitive sequences”

(274 citations to date)

Evolution of CRISPR-Cas9 in prokaryotes

CRISPR loci of S. thermophilus

CRISPR Mediates Microorganism “Immunity”

Type I, Type II and Type III CRISPR Systems

Type I, Type II and Type III CRISPR Systems
Integration of exogenous protospacers by Cas system

Timeline of CRISPR Discoveries

<table>
<thead>
<tr>
<th>Year</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>’Short Regular-Spaced Repeats’ in Bacteria (SRSR)</td>
</tr>
<tr>
<td>2002</td>
<td>First use of the term ‘CRISPR’; Description of ‘CRISPR-associated’ (Cas) genes</td>
</tr>
<tr>
<td>2005</td>
<td>CRISPR spacer sequences are complementary to bacteriophage sequences</td>
</tr>
<tr>
<td>2007</td>
<td>CRISPR sequences can modify bacterial susceptibility to phage infection</td>
</tr>
<tr>
<td>2011</td>
<td>CRISPR-Cas9 work together to attack foreign RNA</td>
</tr>
<tr>
<td>2012</td>
<td>First synthetic ‘Guide RNA’</td>
</tr>
<tr>
<td>2013</td>
<td>Widespread genome editing using CRISPR systems</td>
</tr>
</tbody>
</table>

Timeline of CRISPR Discoveries

Sequence considerations for CRISPR-Cas

Sequence considerations for CRISPR-Cas: The PAM
Use of CRISPR as a research tool

- Buy CRISPR-CAS plasmid. (2 days)
- Clone in target sequence. (1 week?)
- Put in your favorite cells. (2 days)
- Measure effect. (1 day+)

Uses of CRISPR in the lab:
1. What we’d like to do with it

<table>
<thead>
<tr>
<th>Non homologous end joining</th>
<th>Homologous recombination</th>
</tr>
</thead>
</table>
| ![Diagram](image)

Interaction between BRCA1 and 53BP1 at sites of radiation-induced DNA damage

Irradiated nucleus showing immunofluorescent foci of 53BP1 and Brca1 after 10Gy radiation

Use of I-SceI meganuclease to introduce site-specific DNA breaks

Existing Tools for Genome Editing: TALENs and Zinc Finger Nucleases

Alterning the Genome of a mouse is much easier with CRISPR
Altering the Genome of a mouse is much easier with CRISPR

CRISPR/Cas9

Genomic DNA

Donor with Reporter

Point mutation

Detection

Small DNA insertion

Where Will CRISPR End Up On the 'Hype-Cycle'?

Economic
Utility

Statins

Monoclonal
Antibodies

Artificial
Heart

Statins

3D printing

Statins

Small
Regulatory
RNAs

Human
Genome
Project

Human Health applications of CRISPR

Schwarz G et al, Cell Stem Cell, 2013

Questions?

Structure of S. pyogenes Cas9

One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/CAS-Mediated Genome Engineering

Cell 154, 1370-1379, September 12, 2013

Narendra Kuber

Aims

1. To demonstrate CRISPR/CAS as a simple and efficient method for one-step insertion of a short epitope or longer tags in precise genomic locations in a mouse zygote

2. To apply this principle for
   i. generating transgenic mice with conditional alleles and endogenous reporters
   ii. deleting regions up to 700bp in a targeted gene

3. Off-target mutations are rare in targeted ES cells and mice

Background

Zygote → Embryo → Transgenic mouse

Wobus A M , and Boheler K R Physiol Rev 2005;85:635-678

Differentiation

Comparative efficiency of injection techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Percent Competent Zygote (%)</th>
<th>Percent Complete Embryo (%)</th>
<th>Time to Genotype (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-step</td>
<td>80</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>Two-step</td>
<td>70</td>
<td>50</td>
<td>15</td>
</tr>
</tbody>
</table>

• One-step better than two-step

• No difference between cytoplasmic vs. pronuclear injection

Aim 1

One-step insertion of a V5tag at the end of Sox2 gene

ORF – open reading frame
SF, V5F, SR – primers used for genotyping
PCR Genotyping

Western Blot

Sequencing data

PCR genotyping, Western Blot and Sequencing confirm the insertion of the V5 tag at the end of the Sox2 gene.

Aim 1

One-step insertion of a V5 tag at the end of Sox2 gene

Western Blot

Targeted blastocyst

Targeted mES cells

Immunostaining assay using V5 antibody

Aim 1

One-step generation of mCherry reporter in Nanog gene

Southern Blot

ES cell line genome

Aim 1

One-step generation of mCherry reporter in Nanog gene

Confocal microscopy

Mouse genome from injected zygotes

Confocal microscopy

Signal disappears upon differentiation

Confocal microscopy

mCherry expression seen in cells that express Nanog gene

Confocal microscopy

mCherry expression seen in cells that express Nanog gene

Confocal microscopy

Signal disappears upon differentiation
One-step generation of mCherry reporter in Nanog gene

**Aim 1**

- Arrows indicate mCherry negative colonies
- Mosaic expression akin to ZFN and TALEN-mediated targeting

**Confocal microscopy**

Results Summary (1)

- HDR-mediated genome editing can offer targeted reporter gene integration efficiently through CRISPR/CAS-mediated genome editing

Mosaicism remains a challenge

<table>
<thead>
<tr>
<th>Donor</th>
<th>Mosaic/Total targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanog-Cherry</td>
<td>Mouse: 1/7</td>
</tr>
<tr>
<td></td>
<td>ESCs: 2/8</td>
</tr>
<tr>
<td>Oct4-EFGP</td>
<td>Mouse: 1/3</td>
</tr>
<tr>
<td></td>
<td>ESCs: 1/8</td>
</tr>
</tbody>
</table>

Targeted mice or ESCs were identified by FIDP, Southern blot or Sequencing. The frequency of mosaicism in targeted mice was determined by fluorescent reporter or Southern blot analysis.

Similar to ZFN and TALEN-mediated targeting

One-step generation of Oct4-eGFP knockin allele

**Aim 1**

- Southern Blot

One-step generation of Mecp2 Floxed allele

**Aim 2**

- Schematic of targeting sites in Mecp2 gene

- Sequence of a floxed allele

Results with Resequencing in the Endogenous Genes

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Nanog-Cherry</td>
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</tbody>
</table>

Targeted mice or ESCs were identified by FIDP, Southern blot or Sequencing. The frequency of mosaicism in targeted mice was determined by fluorescent reporter or Southern blot analysis.

Similar to ZFN and TALEN-mediated targeting
In-vitro recombinase incubation assay

Aim 2

Deleting a gene using CRISPR/CAS

Deletion events in mice #1, #6 and #8

Transgenic mice DNA

Aim 2

Mutant allele sequences

Deletion events in mice #1, #6 and #8

Results Summary (2)

- CRISPR/CAS-mediated genome editing is a powerful technique to generate transgenic mice with conditional alleles or study effects of deleted genes

Table 2: Conditional Mcep2 Mutant Mice

<table>
<thead>
<tr>
<th>Host genome</th>
<th>Mouse zygote</th>
<th>Human cell line treated with CRISPR/CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal genome derived from a single zygote</td>
<td>Genome obtained from heterogeneous population of cells</td>
<td></td>
</tr>
</tbody>
</table>

DNA repair systems

- Zygote is subjected to fewer DNA damage responses
- Transformed human cell lines may have different and more ways to damage DNA and mediate repair
- DNA plasmid used to transfect cell-lines is long-lived leading to more extensive DSB events

Advantages of CRISPR/CAS

- Principle can be applied to other mammalian species for their genetic modification without the need to establish ES cell-lines
- Cytoplasmic injection technique has similar efficiency compared to pro-nuclear injection technique and is a lot easier to accomplish

Factors

- Host genome
- DNA repair systems
- Duration of exposure to CRISPR/CAS

Mouse zygote

- Zygote is subjected to fewer DNA damage responses
- DNA plasmid used to transfect cell-lines is long-lived leading to more extensive DSB events

Human cell line treated with CRISPR/CAS

- Genome obtained from heterogeneous population of cells

CRISPR/CAS sgRNA was injected into isolated pre-implantation embryos. The zygotes were treated with CRISPR/CAS sgRNA and Cas9 mRNA. Both L2 and R1 sgRNA injection without donor plasmid led to deletion of the region between LoxP sites.

Duration of exposure to CRISPR/CAS

- sgRNA is short-lived causing fewer DSB events
- DNA plasmid used to transfect cell-lines is long-lived leading to more extensive DSB events

Advantages of CRISPR/CAS

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Advantages of CRISPR/CAS

- Principle can be applied to other mammalian species for their genetic modification without the need to establish ES cell-lines
- Cytoplasmic injection technique has similar efficiency compared to pro-nuclear injection technique and is a lot easier to accomplish
Limitations of this study / Future work

- Correlation between sgRNA design and efficiency in targeting the intended genomic region is not completely understood
  - whole genome sequencing approach
- Mosaicism remains a challenge
- Southern blot analysis cannot identify small in-del mutations and thus undermines the frequency of mosaicism
- Higher frequency of DSBs compared to the insertion of intended gene modification may cause issues
Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression


Dharm Patel
Rutgers Biotechnology Training Program
Bunting Laboratory
March 7th, 2014

Current Technologies for Targeted Gene Regulation

- TALE - Transcription activator-like effector proteins
- Zinc Fingers
- RNAi – RNA interference using antisense RNA against transcript and host silencing machinery (dicer, RISC, argonaute)
- shRNA – viral transduction, nuclear expression, stable
- siRNA – liposome/electroporation transfection, high doses required, transient

Modified CRISPR/Cas9 System for Gene Interference

1. Nuclease dead Cas9 protein (dCas9)
2. Small guide RNA (sgRNA) – provides DNA binding specificity

CRISPRi Silences Transcription Elongation and Initiation

Factors that Affect CRISPRi Efficiency

- Specificity of CRISPRi is determined by:
  - PAM (NGG)
  - ≥ 12 bp of sgRNA:DNA stretch
- This is large enough to cover most bacterial genomes for unique sites.
CRISPRi Mechanism

Modulating E. coli Lactose Regulatory Network Using CRISPRi

CRISPRi functions by physically blocking transcription machinery.

Knockdown of essential activators of LacZ expression = decreased ß-gal activity
Knockdown of LacZ repressor, lacI = increased ß-gal activity

Concluding Remarks

Summary
- CRISPRi, composed of dCas9 and a locus specific sgRNA, can efficiently, selectively, and reversibly repress transcription of target genes by physically blocking RNAP in bacterial and mammalian systems.
- Parameters for optimization in bacterial system: PAM + 12 nucleotide base-pairing sgRNA:DNA(non-template)

Technology Outlook
- sgRNA libraries can be used for genome-scale analysis and regulation.
- CRISPRi can be used as a platform to modulate genome site specific biological processes (transcriptional activation, chromatin modification) using dCas9 fusion proteins.

Critiques
- Structural study required to provide details of RNAP blocking.
- Characterization and optimization of dCas9/sgRNA system in mammalian cells.
- RNAi and CRISPRi gene knockdown efficiency

Bacterial and Mammalian Plasmids available on Addgene

Acknowledgements

Dr. Samuel Bunting
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RU Biotechnology Training Program
Using molecular visualization tools to understand protein structure and function

Vikas Nanda, Ph.D.
Robert Wood Johnson Medical School
nanda@cabm.rutgers.edu

These slides are representative images from a software demonstration for the Biotechnology Topics Seminar using the molecular visualization tool – pyMol – to examine the structure of hemoglobin. Students were encouraged to use such tools to examine atomic structures of proteins related to their research.

Three dimensional structure of hemoglobin shows the four protein domains.

Cavities on the surface of the protein often contain bound water molecules (red spheres).

The oxygen binding heme cofactor is tightly bound in a pocket on the surface of each domain.

Each subunit of hemoglobin is made up of eight alpha-helices comprising the globin fold.

Closeup of an alpha-helix.
Separate representations of the peptide backbone (left) and sidechains (right) of the first helix in hemoglobin.

Hydrophobic amino acids are found on one face of the alpha-helix.

Hydrophobic amino acids make up the core of each domain of hemoglobin.

Inter-domain contacts are primarily through electrostatic interactions.
An antifreeze Protein Folds with an Interior Network of More Than 400 Semi-Clathrate Waters

Paper By: Tianjun Sun et al.
Paper Review by: Agnes Yeboah
April 4 2014

Protein Folding
- Most globular proteins have a dry core
  - Aliphatic and aromatic side chains pack together to form a hydrophobic core
- During the folding process, water is released out of core
  - Dewetting mechanism: water evaporates from partially formed core
  - Expulsion mechanism: Partially solvated hydrophobic core; water expulsion
- Overall gain in entropy that powers the folding process

Antifreeze Proteins (AFPs)
- To survive wintry weather, organisms such as fish, insects, and plants have developed antifreeze proteins that inhibit growth of ice crystals in their tissues.
- The AFPs adsorb to the surface of small ice crystals and prevent their growth into large crystals.
- AFP Type 1 proteins
  - Typically alpha helices
  - Typically rich in alanine
  - Ice binding residues typically on surface of helix

Fig. 1: Model of shorthorn sculpin (fish) AFP binding to a secondary prism plane of ice. Refer to: Baardsnes et al., "Antifreeze Protein from Shorthorn Sculpin: Identification of the Ice-Binding Surface". (2001) Protein Science 10, 2566-2576.

Maxi/Relevance
- Maxi
  - Studying one such protein called Maxi from winter flounder, researchers have discovered that the protein has an unexpected structure.
  - Unlike similar antifreeze proteins—and unlike other proteins, generally—Maxi incorporates sheets of hundreds of water molecules into its core.
- Why is this relevant?
  - Maxi’s structure violates the anhydrous-core principle
  - Do we fully understand the mechanism of protein folding?

Maxi Structures
- Structure of Maxi
  - Four helix bundle
  - Highly rich in alanine
- How the capping structure of Maxi makes the helices stay apart
- How a surface representation of Maxi shows minimal interaction between the chains
- How Maxi binds to ice
  - Water network in Maxi
  - Semi-clathrate water structure
  - Positioned waters extending from core of four helices to the surface

Acknowledgement
- Dr Vikas Nanda
- Jose James
- Phil Tedeschi
- Biotechnology Training Program
- Dr Peter L. Davies & Dr. Robert Campbell (Queens University, Canada)
- Pymol structure of Maxi
A brief history of amyloid fibrils

- The term "amyloid" introduced in 1854 by Rudolph Virchow from pathological brain slices stained withiline and sulphuric acid.
- Interests in amyloid bodies grew with classification of clinical symptoms for amyloidosis.
- Process in which soluble proteins form insoluble aggregates that deposit in the extracellular space of tissues and disrupt normal function.
- Microscopic studies showed that all amyloids exhibited "sticky" behavior with polarized light.
- Suggested possibility of a common ordered structure.
- Biochemical study of isolated amyloid fibrils revealed that amyloidosis is caused by a diverse range of proteins (led to discovery of 20 soluble proteins that caused amyloidosis).
- Common structure of amyloids confirmed.
- EM studies on a diverse range of fixed tissue sections show a common structure comprised of a bundle of straight rigid fibrils.
- X-ray diffraction analysis of isolated protein fibrils showed a β-sheet backbone oriented parallel to the fibril axis. Labeled as a Cross-β spine.
- By the late 20th century, researchers were able to create in vitro amyloid fibrils from a wide array of proteins by partial denaturation through chemicals and heat.

The modern understanding of amyloids

- "Effectively all complex proteins have these short segments that, if exposed and flexible enough, are capable of triggering amyloid formation" - David Eisenberg (author).
- Amyloid fibers form from partial unfolding events that expose specific set of short sequences that are intrinsically "sticky".
- Current paper provides atomic detail of the cross-β spine.
- Provides insight into why amyloidogenic sequences are "sticky" and why they are very stable.
- Use structural insights to predict sequences in proteins likely to form a cross-β spine.
  - Identifying the "amyliome".
Dry Interphase critical for amyloid structure

Amyloid Nucleus
Using iPSCs to Model Human Cognitive Disorders
Bonnie L. Firestein, Ph.D.
Department of Cell Biology and Neuroscience
Rutgers University

Induced Pluripotent Stem Cell (iPSC) Technology

- 2006- Takahashi and Yamanaka show the induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by the expression of OCT3/4, SOX2, KLF4, and c-Myc.
- 2007- Takahashi et al. show the induction of pluripotent stem cells from adult human fibroblasts by expressing the same 4 factors.
- 2007- Yu et al. show the induction of pluripotent stem cells from human somatic cells by the expression of OCT4, SOX2, NANOG, and LIN28.

Reprogramming Genes Used To Generate iPS Cells

- Reprogramming genes-transcription factors that have been shown to function in the maintenance of pluripotency in both early embryos and embryonic stem cells
- Oct3/4, SOX2, KLF4, c-Myc: As transcription factors, they increase or decrease the expression of many genes

Reprogramming Genes Used To Generate iPS Cells, Continued

- Fibroblasts are actively dividing cells
- Use a packaging cell line to make replication-incompetent viruses
- Transduce cells with viruses containing the 4 reprogramming genes
- Stem cell colonies appear 21 to 30 days after transduction. Kristina saw putative colonies at 14 days after transduction.
qPCR as assayed on the AB Stem Cell TLDA card

- Total cellular RNA was isolated from my putative iPSC colonies using TriZol.
- mRNA expression of 96 validated genes associated with stem cell pluripotency and differentiation to all three germ layers was analyzed using the Human Stem Cell Pluripotency Taqman Low Density Array (TLDA) fluidic card (Applied Biosystems).
- The expression pattern for my putative iPSCs was then compared to the expression patterns of other cell types.

**Rutgers**

**Generation of NSCs**

- Grow hiPSCs in media supplemented with Noggin (BMP4 inhibitor), withdraw Noggin (morphogen-free pathway), and add FGF (elicits proliferation of NSCs).

**Rutgers**

**Characterization of hiPSCs, hiPSC-derived NSCs, and hiPSC-derived neurons.**

**Schizophrenia**

- Schizophrenia is a serious psychiatric disorder.
- Affects approximately 1% of the population.
- Genetic basis established through twin, adoption, and family studies.
- Significant linkage and linkage disequilibrium of familial schizophrenia to chromosome 1q22 in Canadian families (Brzustowicz et al., 2000; 2002).
- Meta-analysis showed linkage of schizophrenia to 1q22 (Lewis et al., 2003).
- NOS1AP isoform protein levels are altered in specific regions of the brain for patients diagnosed with schizophrenia (Hadzimichalis et al., 2010).
Schizophrenia

• Dendritic abnormalities in the prefrontal cortex (PFC; Black et al., 2004; Garey et al., 1998; Glantz and Lewis, 2000; Kalus et al., 2000; Schultz and Andreasen, 1999)
• Reduction of hippocampal size (Lawrie and Abukmeil, 1998; Nelson et al., 1998; Wright et al., 2000) and altered hippocampal shape (Csernansky et al., 2002).
• At the cellular level, the most consistent change observed is an abnormal synaptic connectivity.

NOS1AP

• Formerly known as CAPON
• C-terminal PDZ domain ligand of neuronal nitric oxide synthase
• N-terminal phosphotyrosine binding (PTB) domain that binds synapsin and the small G protein Dexras1
• 10 exons, ~300 kb genomic extent
• At least three isoforms:
  - “long”: 10 exons, previously in literature
  - “short”: last two exons, produces a 210 aa protein (described in Xu et al. 2005)
  - “new short”: Further truncated isoform

NMDAR-nNOS-NOS1AP

NOS1AP can act to decouple nNOS activity from the NMDA receptor and redeploy nNOS elsewhere in the cell
NOS1AP expression in BA46

Dendrite numbers are decreased in model of schizophrenia

Overexpression of NOS1AP isoforms decreases dendrite branching in hPSC-derived neurons.

Antipsychotics decrease dendrite branching of hPSC-derived neurons in a NOS1AP-independent manner.

NMDAR agonists restore normal branching in hPSC-derived neurons overexpressing NOS1AP-L

Kristina Hernandez

Firestein laboratory

Kristina Hernandez

Dr. Yangzhou Du
Dr. Damien Carrel
Dr. Norell Hadzimichalis
Dr. Michelle Previtera
Meera Trivedi
Natasha Dudzinski

Collaborators
Dr. Linda Brzustowicz
Dr. Jim Millonig

FUNDING
NSF, MOD, NARSAD
NIH (Brzustowicz)
Modeling schizophrenia using human induced pluripotent stem cells

Ilija Melentijevic
Biotechnology Training Program Rutgers

Schizophrenia (SCZD)
- Affects 1% of the population
- Has a strong genetic component with 80-85% heritability
- Post mortem studies have revealed many cellular and molecular characteristics
- Underlying mechanisms still unknown

Reprogramming SCZD Fibroblasts into neurons

SCZD hiPSCs had no problem differentiating

Decreased neural connectivity

Reduced rabies trans-neuronal tracing in SCZD neurons
Works by labeling retrograde transmission with RFP
Modified ENVA rabies that needs TVA receptor to infect

Loxapine improved connectivity

Other antipsychotics Clozapine, olanzapine, risperidone and thioridazine had no effect
**SCZD had reduced neurites and PSD95**

- Reduced ratio of PSD95 to MAP2B
- Reduced PSD95 density (not significant)
- No change in other synaptic proteins (SYN, VGLUT1, GLUR1, VGAT, and GEPH)

**Action Potential**

**Electrophysiology**

SCZD had normal action potentials, and spontaneous excitatory and inhibitory synapse activity

**SCZD Altered gene expression**

596 genes had altered expression
25% of those genes implicated in Schizophrenia

**NRG1**

- NRG1 is commonly overexpressed in SCZD
- Only found to be expressed in SCZD neurons, not iPSCs or NPCs
- Loxapine increased NRG1 expression as expected

**Loxapine on gene expression**

- Increased expression of glutamate receptors GRIK1, GRM7 and GRIN2A
- Reduced expression of ADCY8, PRKCA, WNT7A and TCF4
- Establishes model for studying effects of antipsychotics on gene expression in live SCZD neurons
Summary

Predicted Aspects of SCZD Cellular Pathology

<table>
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<th>Positive Findings in SCZD hiPSC Neurons</th>
<th>Negative Findings in SCZD hiPSC Neurons</th>
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- Improved neuronal connectivity with Loxapine (10 µM)
- No change in EPSCs or the amplitude, frequency or spontaneity of spontaneous calcium transients

Critiques

- Generated neurons looked bad
- Only found an effect with one antipsychotic
- Didn't provide information on how they controlled the patients

Acknowledgements

- Dr Firestein
- Ana Rodriguez
- Dr Yarmush
- Dr Stock
- Biotechnology Training Program
SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients

Trevan Locke

PMDS background

- Phelan-McDermid Syndrome is a gene disorder resulting from the deletion of a portion of chromosome 22.
- Characterized by developmental delay, intellectual impairment, and often autistic-like behavior.
- Almost all cases involve deletion of SHANK3, a gene that encodes a scaffold protein that connects membrane bound receptors to the actin cytoskeleton.

PMDS patient selection

Differentiation

Removal of growth factors and induction of neuronal fate

Introduction of fluorescent proteins through lentiviruses

PMDS and control cells co-cultured on astrocytes

Introduction of neuronal medium followed by isolation of rosettes.

Neuronal Markers present in differentiated cells

Synapse Review

- Post-synaptic density (PSD) concentration of receptors and other proteins at the post-synaptic membrane
- EPSC makes cell more likely to fire action potentials
- IPSC makes cell less likely to fire action potentials
- Puncta-clusters of proteins within PSD, show up as bright dots with fluorescent imaging
PMDS Neuron Characterization

• PMDS neurons showed increased input resistance and decreased amplitude and frequency.

• Similar action potential, resting membrane potential, and capacitance

Inhibitory synaptic transmission was identical to control neurons

SHANK3 Expression

• As mentioned, PMDS neurons have decreased levels of SHANK3 expression.

SHANK3 Expression

• Transduced SHANK3 lentivirus restores AMPA-EPSC’s in all cells and NMDA-EPSC’s in 43% of cells.

Treatment with IGF1 restores deficits
IGF1 Treatment

- Difference between SHANK3 and IGF1 deficit restoration
- IGF1 actually reduces SHANK3 expression, but compensates by increasing PSD95 expression
- Changes in NMDA decay rate indicative of this difference

Summary

- Loss of SHANK3 implicated in onset of PMDS.
- Patient derived iPSC’s reprogrammed to neurons to study gene expression and synapse activity.
- PMDS neurons have decreased excitatory transmission.
- Transduced SHANK3 and administered IGF1 are shown to recover synaptic deficits.

Critique

- Controls lacking in SHANK3 transduction experiments.
- There were only two patients used.
- Co-culture model allows control and PMDS cell lines to be studied under identical conditions in addition to showing cell autonomy.

Acknowledgements

- Dr. Bonnie Firestein
- Ana Rodriguez
- Ilija Melentijevic
- Rutgers Biotechnology Training Program

Additional References


Questions?
Mechanobiology in Stem Cells

Professor Prabhas Moghe
May 2, 2014

Stem Cells for Regeneration: Steering Cell Differentiation

**Human Mesenchymal Stem Cells (hMSC)**
- Self-renewing cell population that can differentiate into a variety of cell types: osteoblasts, chondrocytes, myocytes, adipocytes, etc...
- Stem cell differentiation affected by biomaterials and/or soluble factors
- Ultimate goal is design microenvironments that steer stem cell differentiation
- Cues that control the stem cell decision-making process need to be elucidated

---

**Cell morphological characteristics are influenced by complex signaling mechanisms**

**Stem cell differentiation is controlled by cell spreading area**

**Stem cell differentiation is controlled by substrate elasticity**

**Signaling Between the Mechano-responsive Cytoskeleton and Nucleus**
Matrix-directed Differentiation is Mediated by the Nucleus

Stem cell differentiation is stiffness-dependent

- Lamina-A increases
- RARG and YAP1 translocate to nucleus

Discher et al., Science, 2013

Capturing differences in mechanosensitive nuclear proteins

High-content analysis of SC-35 domains yield morphologically distinguishable cell features

SC35 is sensitive to the osteogenic differentiation pathway

In Situ Mechanotransduction Via Vinculin Regulates Stem Cell Differentiation

Presenter: Paulina Krzyszczyk
May 2, 2014

Michelle Sempkowski
May 2, 2014
**Directing stem cell fate on hydrogel substrates by controlling cell geometry, matrix mechanics and adhesion ligand composition**

Junmin Lee, Amr A. Abdeen, Douglas Zhang, Kristopher A. Killian

Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, IL, USA

Michelle Sempkowski
May 2, 2014

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**Mechanotransduction in MSC differentiation**

- Mechanotransduction shown to play an important role in stem cell differentiation, dependent on BOTH the mechanics and composition of the microenvironment

- A number of studies have

  - Substrate
  - Components
  - Cells

---

**The gap**

- **Current research**: majority focuses on varying ONE physical cue and studying the corresponding biological effects of MSC differentiation
- **Reality**: MSC fate is most likely controlled by a combination of geometry, mechanics and ECM composition

The major gap is a system that combines all of these cues and more closely mimics the in vivo microenvironment to be able to “fine-tune” differentiation outcomes

---

**Objectives**

1. Explore the physical and biochemical cues that guide MSCs towards adipogenesis and neurogenesis by manufacturing micropatterned soft hydrogels (<1 kPa) with:
   - Variable geometry
   - Variable ECM proteins deposited on the surface

2. Assess the expression of key markers during differentiation using immunofluorescence, real-time PCR, and Oil Red O staining (adipogenesis only)
   - Adipogenesis: p-par y (IF); C/EBPα and LPL (rtPCR)
   - Neurogenesis: beta3 tubulin and MAP2 (IF and rtPCR)

3. Explore the translatable aspects of this work to the in vivo environment using a pseudo-3D hydrogel

---

**Soft hydrogel fabrication and single cell patterning**
Soft hydrogel fabrication and single cell patterning

- After seeding cells on fibronectin coated surfaces, they confirmed that:
  - Cells substantially adhered to the patterned regions
  - Patterned cell heights greater than those unpatterened
  - Average patterned cell area approximately pattern size; unpatterened cells of variable spread area
  - Patterned cells viable and restricted to the islands for 13 days

MSC differentiation on unpatterned hydrogels

- Unpatterened, fibronectin-coated hydrogels show mix of cells expressing markers associated with adipogenesis (p-par g) and neurogenesis (beta3 tubulin)

MSC differentiation on micropatterned hydrogels

 MSC differentiation on micropatterned hydrogels with different matrix proteins

- In vivo:
  - Adipose matrix contains collagen, laminin and fibronectin
  - Brain tissue matrix contains hyaluronan, collagen and laminin

MSC differentiation on micropatterned hydrogels with different matrix proteins

- MAP2

MSC differentiation on micropatterned hydrogels with different matrix proteins

- Oil Red O
The combined influence of geometric cues and matrix proteins

MSC differentiation in pseudo-3D microenvironments

Conclusion

- Fate of stem cells on hydrogels in the presence of differentiation media has been considered controversial
- Here they show in 2D and 3D:
  - Shape alone:
    - Round MSCs → higher levels of adipogenic markers
    - Spread MSCs → high levels of neurogenic markers
  - Matrix proteins alone:
    - MSCs cultured on Fn → elevated adipogenic markers
    - MSCs on Cn → elevated neurogenic markers
  - Combination:
    - Round MSCs on Fn, Ln, or Fn/Ln → adipogenic markers
    - Round MSCs on collagen, or spread on any protein → neurogenic markers
- Phenomena in vivo are intimately connected with in vitro fate
- “Combining these cues can maximize differentiation outcomes”

Critiques

- Type of neurons not specified and functional tests lacking
- No comments on mechanisms of mechanotransduction
- Hyaluronic acid important component of brain tissue in vivo, but not used here for stimulating neurogenesis
- Never reached 100% differentiation for any pattern, or combination of proteins, ever! What kind of fine tuning is this?!
- How can they say 2000 μm² area in 3D was comparable to 2D data that studied the effect of area? They didn’t even test that area

Acknowledgements

- Dr. Moghe
- Perry Yin
- Paulina Krzysczyk
- Biotechnology Training Program
Questions?
In Situ Mechanotransduction Via Vinculin Regulates Stem Cell Differentiation
Holle et al., 2013
Presenter: Paulina Krzyszczyk
May 2, 2014

Stiffness-Mediated Differentiation Mechanisms
- Actin-myosin contraction → RhoA/ROCK → Differentiation
- Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ)

Upstream signaling cascades are unknown

Force-Induced Protein Stretch
- Focal adhesion proteins stretch with ↑ substrate stiffness
- Buried sites become exposed

Molecular Strain Gauge
- Talin
- Vinculin

Modified from del Rio et al., 2009
Jaalouk & Lammerding, 2009

Study Hypothesis
A molecular strain gauge within focal adhesion proteins may bury a biochemical cue that can induce mesenchymal stem cell differentiation

What is vinculin’s role in mechanotransduction and differentiation?

Main Methods
- Cell Substrate
  - Polyacrylamide hydrogels (1 kPa, 11 kPa or 34 kPa)
  - Collagen I crosslinked
- Cells
  - hMSC from Lonza
  - Differentiated over the course of 6 days
- Vinculin Knockdown (Vinc KD)
  - siRNA transfection (transient)

Vinculin Knockdown and MyoD Expression

Vinc KD successful
Less MyoD in Vinc KD

60% reduction in MyoD signal in Vinc KD
Differentiation Marker Expression

- Myogenic
- Osteogenic

Expression of myogenic markers in Vinc KD
Same expression levels of osteogenic markers

Vinculin and Durotaxis

Firm = 11 kPa
Soft = 1 kPa

Vinculin must be present for timely directed migration

Inaccessible MAPK1 Binding Site on Vinculin

465 kinase binding sites in adhesome

Co-localization of Vinculin and pMAPK

Vinc and pMAPK co-localized

Rescue of Myogenesis by Vinculin Add Back

MAPK1 Mutation Decreases MyoD Expression
MAPK1 and Mechanosensitive Differentiation

↑ pMAPK1 on stiffer matrices

MAPK1 Inhibitors and MyoD Expression

Inhibitors did not significantly affect
- Cell morphology
- Vinculin expression
- Vinculin assembly
- Cell area

Only signaling is altered

MAPK1 Inhibitors and MyoD Expression

Other Effects of Vinculin Knockdown

Cell viability

Focal adhesion structure

Focal Adhesion Strength

Spinning disc assay: Measures adhesion strength by applying radial shear

Traction force microscopy: Measures cell-generated traction forces by deformation of substrate

Summary

Linked the role of in situ change in vinculin with myogenesis in hMSCs due to the mechanical environment

- Vinculin KD impaired
  - myogenesis (NOT osteogenesis)
  - durotaxis
- Vinculin KD did not impair
  - Cell viability
  - Focal adhesion structure/strength
- Inaccessible MAPK1 site on vinculin identified
- MAPK1 site necessary for MyoD rescue
- MAPK1 inhibition ↓ MyoD expression

First evidence that a force-sensitive focal adhesion protein can regulate stem cell fate

Proposed Mechanism of Talin/Vinculin/MAPK1 cascade
**Discussion**

- Vinculin has previously shown to affect adhesion strength and traction
  - Different roles in terminally differentiated versus stem cells
  - Serum level requirements for hMSCs may affect adhesion strength

- MAPK independent alternatives to rescuing MyoD expression?

- MAPK1 signalling
  - involved in mechanically induced myogenic differentiation
  - involved in chemically induced osteogenic differentiation

**Critique**

**Pros**

- Comprehensive investigation of myogenic differentiation controlled by substrate stiffness, vinculin and MAPK1 site

**Suggestions**

- Include more results/explanation regarding lack of osteogenic differentiation
  - Other lineages
- Connection between Vinc → ROCK → YAP/TAZ → MyoD signaling
- How is focal adhesion strength not affected if we see delayed durotaxis in Vinc KD cells?

**Potential Future Directions**

- Is this mechanism the same in other stem cells?
- What is the effect of varying the ECM protein?
- What other molecular strain gauges play a role in differentiation?

**References**


**Acknowledgements**

- Dr. Prabhas Moghe
- Perry Yin
- Michelle Sempkowski
- Biotechnology Training Program
Course Description

This course introduces and outlines the fundamentals of "technology entrepreneurship" and introduces a framework for identification of high-potential, technology-intensive, commercial opportunities, assessment of competition, innovation protection, competitive assessment, gathering required resources (human and financial), and readying the innovation to commercializable product.

Through a collection of lectures and readings that address high-growth ventures in information technology, electronics, life sciences, biotechnology and related industries, this course places a specific focus on commercialization derived from scientific and technological research.

Students are expected to apply each of the concepts learned to a “Team Venture” (specific innovations provided by the instructor and students from Innovative Senior Design program use their assigned research projects), and submit written responses/present to the class regarding various aspects of its commercialization. At class conclusion, presentation of a Team Venture pitch is made to a team of "judges" comprised of subject matter experts from representative commercialization disciplines.

Knowledge gained will be useful to those having little or no pre-existing entrepreneurial or technology commercialization experience.

Instructor(s)

As the course is comprised of sessions focused upon unique individual aspects of the commercialization process, several instructors are potentially involved in its delivery as follows:

- Dr. Francois Berthiaume, Associate Professor of Biomedical Engineering
- Susan Engelhardt, Executive Director, CIVET
- Dr. Stavroula Sofou, Associate Professor of Chemical and Biochemical Engineering
- Dr. Martin Yarmush, Distinguished Professor of Biomedical Engineering, Mary and Paul Monroe Chair in Science and Engineering, Director of CIVET, Senior Associate Dean for Research Development and Communication

Where no expertise is resident within the Rutgers schools, outside instructors (with affiliation/history with the Rutgers schools) are recruited to teach specific sessions. As well, to address certain business or legal-intensive topics, senior Faculty Member instructors are recruited from Rutgers University schools including Rutgers Business School, Rutgers Law School, and others to ensure that the required expertise is represented.

Guest speakers are invited to give specialized presentations, as appropriate.
Course Objectives
1. Foster understanding of key technology commercialization concepts and successful entrepreneurship
2. Develop the ability to assess technologies for their commercialization potential through team-based projects based upon venture team innovation
3. Actualize the steps that a technology goes through in the journey from the laboratory to the market
4. Explore the legal landscape for commercialization addressing intellectual property protection, licensing, etc.
5. Develop the ability to communicate value of the innovation through written and verbal means.

Course Outline
The course follows a six stage entrepreneurial process as follows:

- Analyze the Opportunity
- Protect the Innovation
- Develop the Plan & Set Up the Company
- Market the Opportunity
- Acquire (Financial) Resources
- Implement, Scale & Harvest the Venture

and provides the students with the knowledge and perspective to:

- Innovate and create the vision
- Prepare an industry analysis
- Analyze the market
- Draft the business model
- Determine best method of protection
- File necessary documentation
- Begin business plan development
- Set up the company
- Create the marketing plan
- Build “The Pitch”
- Secure early stage and growth funding
- Implement the business model
- Manage the team
- Exit the venture

Within coverage of these topics areas, we build upon the following critical skills for entrepreneurial success:

- Opportunity evaluation
- Strategic thinking
- Art of selling, persuasion and motivation
- Oral and written communications
- Basics of start-up legal concepts
- Basics of start up finance and accounting
The course consists of lecture with extensive participation between students and the instructor. Concepts are inter-mingled with practical applications whereby students are challenged to apply an academic concept to real-world entrepreneurial context.
Assignments
Throughout the course, there are readings assigned and discussions related to those readings during subsequent classes. For some of these reading assignments, written responses to questions will be required.

In addition to the class readings, students will be assigned to teams to work on a Team Venture provided by the instructor. (At least) the following class project(s) will be based upon the assigned Team Venture:

- Evaluation of the technology relative to reality/ease of commercialization and presentation of results
- Assessment of (prior) intellectual property landscape
- Identification, segmentation and quantification of anticipated market for venture technology
- Development and presentation of a 15 minute elevator pitch for marketing of the product/service to potential investors, partners, etc.

Prerequisites
Undergraduate senior or junior status or graduate student populations who seek to understand the innovative process and its relationship to successful entrepreneurship.

Textbook(s)
There are no textbooks required for this course. Readings are provided throughout the sessions.

Grading Criteria (3 Credit Registrants)
- Overall Class Attendance, Contribution and Discussion  20%
- Team Assignments       30%
- Individual Assignments/Presentations    20%
- Final Student Presentations     20%
- Final Essay     5%
- Attendance at monthly CIVET seminars/alternative assignments  5%

Note that class attendance is mandatory. Each student is allowed one unexcused absence and in the event that he/she is absent two or more times, he/she will forfeit 10% of the grade allocated for class contribution/participation (equal to one letter grade). Students are expected to come to class having read the assigned material, completed the assignment, and well-prepared to engage in dialogue regarding the assigned material. All reading and other preparatory assignments must be completed by their due date(s).

Note as well that Graduate students may be given additional assignment(s) throughout the course.
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<tr>
<th>Date</th>
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<th>Session Objective</th>
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| 1/22/2014 | 1   | **Introduction:** Familiarize the student with course logistics, technology entrepreneurship and the nature of innovation.  
- Course overview  
- Entrepreneurship defined  
- Emerging technologies  
- Profile of an entrepreneur  
- Entrepreneurial types/teams  
- Entrepreneurial risks  
- Staged entrepreneurial process |
| 1/29/2014 | 2   | **Analyze the Opportunity:** Innovate and Create the Vision  
- Observation, problem and need identification  
- Needs filtering  
- Ideation and brainstorming  
- Concept screening  
- Importance of documentation  
- Review of venture project technologies and team assignments  
**Guest Lecturer: Dr. Timothy Maguire, Rutgers and VascuLogic** |
| 2/5/2014  | 3   | **Analyze the Opportunity:** Innovate and Create the Vision (continued)  
- Innovation defined  
- Innovation types  
- Innovation frameworks  
- Initial innovation assessment |
| 2/12/2014 | 4   | **Analyze the Opportunity:** Analyze the Market and Build a Plan, Prepare Industrial Analysis  
- Market analysis and planning  
- Market segmentation  
- Prepare a competitive analysis  
**Guest Lecturer: Rutgers University Librarian(s)-postponed** |
| 2/19/2014 | 5   | **Analyze Competitive Position, Develop the Business Model, Outline the Business Plan**  
- Porter’s 5 Forces  
**Guest Lecturer: Social Media, Ken Winell** |
| 2/26/2014 | 6   | **Market the Opportunity:** Create a Marketing Plan  
- Marketing plan essentials: product, pricing, place, promotion  
- Creating/implementing a brand |
| 3/5/2014  | 7   | **Protect the Innovation:** Determine best protection method, File necessary documentation  
- IP protection overview  
- Patents defined  
- Trade secrets defined  
- Copyrights defined  
- Trademarks defined  
- Strategic alliances and licensing agreements  
**Guest Lecturer: Dr. John Kettle, Rutgers University** |
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<td>• Operational agreements</td>
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<td>• Exiting the venture</td>
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<td>4/16/2014</td>
<td>12</td>
<td>Work on Pitch Presentations</td>
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<tr>
<td>4/23/2014</td>
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<td>Guest Speakers: The Entrepreneurial Experience. TBD</td>
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<td>4/30/2014</td>
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<td>Final Pitch Presentations</td>
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COURSE OFFERING FOR SPRING 2014

BIOENGINEERING IN THE BIOTECHNOLOGY AND PHARMACEUTICAL INDUSTRIES: FUNDAMENTAL AND REAL WORLD PERSPECTIVES

(This course is cross-listed in Chemical and Biochemical Engineering and Biomedical Engineering departments)

Course Directors: Dr. Jessica Molek, Dr. Lawrence Sasso, and Dr. Martin Yarmush

Course will be offered on Wednesday 5:00-8:00 PM

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. 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.

Lecture Topics:
- Drug discovery
- Cell line development
- Monoclonal antibody production
- Virus and mammalian cell culture
- Downstream purification
- Biologics formulation
- Manufacturing of biologics
- Single use technologies
- Regulatory considerations
- Vaccine production
- Regenerative medicine
- Biomaterial design
- Cellular therapeutics
- Tissue engineering

Speakers from the following companies:
- Biozeen
- BristolMyersSquib
- Celgene Cellular Therapeutics
- Chromocell
- GlaxoSmithKline
- Hurel
- Inovio
- Janssen
- MedImmune
- Merck & Co.
- Percivia

A unique course offering that provides students with a chance to learn directly from industrial experts.

For more information, please contact Dr. Jessica Molek at 610-270-6067 or jessica.r.molek@gsk.com
NIH Website for Under Represented Minorities

**Training Program:** Biotechnology Training Program

**Institution:** Rutgers, The State University of New Jersey and the University of Medicine and Dentistry of New Jersey

**Program Director:** Martin L. Yarmush, MD, PhD  
732-445-4500 x6203  
yarmush@rci.rutgers.edu

**URM in Training Program:** 5 of 14 (36%)

**URM in Most Recent Entering Class:** 2 of 6 (33%)

**Recent Trends:** In the last 4 years we have recruited 8 URM to the Biotechnology Training Program. In the last three years, 3 URM students graduated with PhD degrees. In the 20 year history of the program we have consistently had between 23-38% URM representation.

**Most Successful Strategies for Recruitment and Retention:** We personally partner with the Rutgers Graduate School-New Brunswick (GSNB) which leads initiatives to recruit, retain, and graduate excellent graduate students from diverse backgrounds. GSNB has an excellent record of success in broadening participation in the STEM disciplines. These initiatives include:

- Exhibit at professional society meetings and national student research conferences
- Visit feeder schools
  - NJ colleges
  - Minority Serving Institutions nationwide
- Coordinate network of faculty, graduate student, postdoc ambassadors
- Participate in consortia dedicated to broadening participation
- Mine databases to identify prospects and share with Graduate Program Directors
- Host undergraduate summer research program, RISE (Research In Science and Engineering), [http://rise.rutgers.edu](http://rise.rutgers.edu)
- Host Winter Forum for prospects from NJ colleges
- Admissions:
  - Help match recruits with most appropriate program(s)
  - Identify external funding for diversity candidates
- Develop transitional pathways to PhD
- Articulated MS/PhD Bridge – fund one STEM student/year
- Develop best practices for mentoring and academic support (e.g. flexible curriculum, peer tutoring)
- Coordinate professional development activities for diverse cohort

Another very successful approach is our constant contact with the graduate directors of the participating graduate programs. During the recruiting season, we make the graduate directors aware of the open slots in the Biotechnology Training program, and challenge them to help fill them with appropriate candidates, especially URM candidates.
NIH Website for Under Represented Minorities

Diversity Initiatives:

**NSF Northeast Alliance for Graduate Education and the Professoriate, [http://www.neagep.org/](http://www.neagep.org/):** Partnership of 10 research-intensive institutions that develop mechanisms to recruit, support, and mentor underrepresented minority students interested in PhDs and academic careers in the sciences, technology, engineering, or math (STEM). The University of Massachusetts-Amherst, as the lead institution, teams with Rutgers and Boston University, MIT, Penn State, and the land grant colleges of the New England states. Specified minority-serving institutions serve as potential feeder schools.

**NSF Innovation through Institutional Integration (I3) – 5 year grant to strengthen science, technology, engineering and mathematics education.** A new Graduate Innovation and Integration Center will assume responsibility for (a) extending curricula and practices developed by IGERT training grants to all STEM graduate programs and (b) preparing undergraduates for advanced training through undergraduate research experiences and graduate school transition programs.

**The National Name Exchange, [http://www.grad.washington.edu/nameexch/national/](http://www.grad.washington.edu/nameexch/national/):** Consortium of 30 nationally recognized research institutions which collect and exchange the names of their underrepresented minority undergraduates planning graduate study. Along with Rutgers, participating institutions include Harvard, MIT, Princeton, Stanford, Cal Tech, a number of campuses in the UC system, and several other universities. GSNB mines the data and compiles a discipline-specific list for each graduate program.

**The National Physical Sciences Consortium (NPSC), [www.npsc.org](http://www.npsc.org):** Ph.D.-track fellowship program in the physical sciences and engineering. Rutgers has access to a database of nominees, sorted by disciplinary area.

**Graduate Education for Minorities (GEM), [https://was.nd.edu/gem/gemwebapp/](https://was.nd.edu/gem/gemwebapp/):** Network of universities and companies that partner to provide opportunities for underrepresented minority students to earn fellowships for M.S. and PhD programs in the sciences and engineering. GSNB mines the data and compiles a discipline-specific list for each graduate program.

**McNair Program, [www.trioprograms.org/clearinghouse/](http://www.trioprograms.org/clearinghouse/)**

McNair facilitates access to higher education for low income, first-generation college students and students with disabilities. GSNB attends the National McNair conference, mines the data from the national McNair clearinghouse and shares with graduate programs, and partners with our own Rutgers McNair program.

**Student research conferences and professional society meetings:** Examples include the Annual Biomedical Research Conference for Minority Students (ABRCMS, [www.abrcms.org](http://www.abrcms.org)), National Society of Black Physicists/Hispanic Physicists (NSBP/NSHP), Society for Advancement of Chicanos and Native Americans in Science (SACNAS), Historically Black College and Universities Undergraduate Programs (HBCU-UP), California Forums for Diversity, national McNair conference, and the Nat’l Organization of Black Chemists & Chemical Engineers (NOBCChE). The Graduate School also facilitates recruitment at student poster session components of professional society meetings; some such societies have Minority Affairs Committees.
Annual Symposium
AGENDA

Continental Breakfast ........................................................................................................ 9:30 am

Introduction ..................................................................................................................... 10:00 am

Dr. Martin Yarmush

Keynote Address .............................................................................................................. 10:30 am

Dr. Scott A. Banta
Associate Professor
Department of Chemical Engineering
Columbia University

Poster Presentation ........................................................................................................ 11:30 pm

Lunch ............................................................................................................................... 12:30 pm

Poster Awards and Closing Remarks ................................................................. 1:30 pm
Dr. Scott A. Banta graduated with his PhD in Chemical and Biochemical Engineering under Dr. Stephen Anderson from Rutgers University in 2002, where he was also a Biotechnology Training Program Fellow. He then completed a postdoctoral training position at Harvard Medical School with Dr. Martin Yarmush before becoming an assistant, and now associate professor, at Columbia University in the Department of Chemical Engineering. He has over 50 peer-reviewed publications, and has applied for several patents in recent years.

Dr. Banta’s research at Columbia focuses on using protein and metabolic engineering methods to solve problems in biotechnology, including the engineering of protein conformational changes and tissue-specific peptides for targeted drug delivery. He is also interested in research integrating protein engineering and electrochemistry for biosensor development and improving enzyme functionality in biofuel cells. Today, he will speak both about his research and the developing business pursuit relating to the biofuel project.
<table>
<thead>
<tr>
<th>Student</th>
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<tbody>
<tr>
<td>Alvin Chen Biomedical Engineering</td>
<td>Portable Image-Guided Device for Automated Venipuncture</td>
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<tr>
<td>Kathryn Drzewiecki Biomedical Engineering</td>
<td>Characterization of Thermoreversible Collagen for Free-Form Hydrogel Applications</td>
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<tr>
<td>Kate Fitzgerald Biomedical Engineering</td>
<td>The Role of Brain-Derived Neurotrophic Factor in Modulating Neuronal Network Dynamics</td>
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<tr>
<td>Mehdi Ghodbane Biomedical Engineering</td>
<td>A Microfluidic Device for Multiplexing Miniscule Volumes of Cerebrospinal Fluid</td>
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<td>Salim Ghodbane Biomedical Engineering</td>
<td>Mechanical Characterization of Lyophilized Type I Collagen Dispersions from Various Sources</td>
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<tr>
<td>Andrea Gray Biomedical Engineering</td>
<td>Effect of Local Anesthetics on Human Mesenchymal Stromal Cells</td>
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<td>Kristina Hernandez Neuroscience &amp; Cell Bio</td>
<td>NOS1AP Regulates Synapse Formation and Synaptic Strength in Rat Cortical Neurons</td>
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<td>Jake Jacobs Molecular Biology</td>
<td>Sap1 Stalled Replication Forks Guide the Integration of LTR Retrotransposons in Schizosaccharomycespombe</td>
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<tr>
<td>Jose James Biomedical Engineering</td>
<td>Relating Tropomyosin Structure and Stability to Shellfish Allergy</td>
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<tr>
<td>Paulina Krzyszczyk Biomedical Engineering</td>
<td>Growth Factor Release from Mesenchymal Stem Cells Encapsulated in PEGDA Hydrogels</td>
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<td>Narendra Kuber</td>
<td>Pharmacology</td>
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<tr>
<td>Serom Lee</td>
<td>Biomedical Engineering</td>
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<td>Trevan Locke</td>
<td>Chemical Engineering</td>
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<tr>
<td>Frank Macabenta</td>
<td>Cell &amp; Developmental Bio</td>
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<tr>
<td>Ileana Marrero-Berrios</td>
<td>Biomedical Engineering</td>
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<tr>
<td>Adriana Martin</td>
<td>Pharmacology</td>
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<tr>
<td>Ilija Melentijevic</td>
<td>Molecular Biosciences</td>
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<td>Sarah Misenko</td>
<td>Biochemistry</td>
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<td>Antoinette Nelson</td>
<td>Biomedical Engineering</td>
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<tr>
<td>Jillian Nguyen</td>
<td>Neuroscience &amp; Cell Biology</td>
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<td>Dharm Patel</td>
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<td>William Pfaff</td>
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<td>Ana Rodriquez</td>
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<td>Michelle Sempkowski</td>
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<td>Philip Tedeschi</td>
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<td>Gabriel Yarmush</td>
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<td>Agnes Yeboah</td>
<td>Biomedical Engineering</td>
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<td>Perry Yin</td>
<td>Biomedical Engineering</td>
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</table>
**Academic Positions**

Scott Banta  
Associate Professor of Chemical Engineering, Columbia University

Maura Collins  
Associate Professor of Biology, Worcester State University

Joseph Freeman  
Assistant Professor of Biomedical Engineering, Virginia Tech

Joe Ledoux  
Associate Professor of Biomedical Engineering, Georgia Inst of Technology

Elizabeth Manheim  
Assistant Professor, Reproductive Med, Weill Cornell Medical College

David Odde  
Professor of Biomedical Engineering, University of Minnesota

Elizabeth Powell  
Associate Professor of Anatomy and Biology, University of Maryland

Mark Riley  
Professor and Department Head of Agriculture and Biosystems

Constance Schnall  
Professor of Chemical and Environmental Engineering, University of Toledo

Deana Thompson  
Assistant Professor of Biomedical Engineering, RPI

**Industrial Positions**

Carlos Aparicio  
Managing Partner, Chief Operating Officer, ImmunoSite Technologies

Tom Brevia  
Senior Research Scientist, Celgene Cellular Therapeutics

Ramona Lloyd  
Vice President, GPC Biotech

Deena Oren  
Manager, Structural Biology Resource Center, Rockefeller University

Colette Ranucci  
Director, Merck

Gregory Russotti  
Senior Director, Process Development Celgene

Kristi Schmalenberg  
Staff Scientist and Johnson & Johnson

Srikanth Sundaram  
Senior Director, Pharmaceutical Development Eagle Pharmaceuticals

Eric Wallenstein  
Research Scientist, Centocor/Johnson & Johnson

Ken Valenzano  
Vice President Pharmacology, Amicus Therapeutics