4th Annual Texas A&M University
ENG-LIFE WORKSHOP
At the Interface of Engineering and Life Sciences

“Biomanufacturing and Synthetic Biology”

Friday, April 14, 2017
8:00 a.m. – 3:00 p.m.
Memorial Student Center (MSC), Room 2300 C,D,&E

Organizing Committee

Arum Han
Committee Chair
Dwight Look College of Engineering
Paul Hardin
College of Science
Arul Jayaraman
Dwight Look College of Engineering

Allison C. Rice-Ficht
College of Medicine
Won-Bo Shim
College of Agriculture and Life Sciences
C. Jane Welsh
College of Veterinary Medicine & Biomedical Sciences
Welcome!

We would like to welcome you to the 4th Texas A&M University ENG-LIFE Workshop: At the Interface of Engineering and Life Sciences. The purpose of this workshop is to promote multidisciplinary interaction and scientific communication in the field of engineering and life sciences. For this year’s workshop, for the first time, we have set a thematic area, Biomanufacturing and Synthetic Biology. This reflects the growing interest in this emerging area from the campus community. However we are not limited to this topic this year, and still would like to provide a venue where people from all disciplines can come and discuss new ideas. This event will also offer a venue for graduate and undergraduate students to gain valuable experience by presenting their latest research results as well as interacting with fellow students and prominent researchers from Texas A&M University. We hope that you all enjoy this workshop.

Sincerely,

The 2017 Symposium Organizing Committee
ACKNOWLEDGEMENTS

Symposium Organizing Committee

Arum Han
Associate Professor (Committee Chair)
Department of Electrical and Computer Engineering

Arul Jayaraman
Ray Nesbitt Professor (Committee Chair)
Department of Chemical Engineering

Allison C. Rice-Ficht
Senior Associate Vice President for Research
Regents Professor, Department of Molecular and Cellular Medicine
Director, Center for Microencapsulation and Drug Delivery

Paul Hardin
Distinguished Professor and John W. Lyons Jr. ’59
Department of Biology

C. Jane Welsh
Assistant Dean for Graduate Studies, College of Veterinary Medicine
Professor, Department of Veterinary Integrative Biosciences
Chair, Texas A&M Institute for Neuroscience (TAMIN)

Won-Bo Shim
Professor
Department of Plant Pathology & Microbiology

Sponsors

Division of Research
College of Medicine
Dwight Look College of Engineering
<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:15 - 8:45</td>
<td>Registration and Breakfast</td>
<td></td>
</tr>
<tr>
<td>8:45 - 9:00</td>
<td>Opening Remarks</td>
<td><strong>Dr. Glen A. Laine</strong>, Vice President for Research</td>
</tr>
<tr>
<td>9:00 - 9:40</td>
<td>Opening Keynote</td>
<td><strong>New collaboration opportunities to modernize pharmaceutical manufacturing and translation of TAMU discoveries from bench to bedside</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Mansoor Khan</strong>, College of Pharmacy</td>
</tr>
<tr>
<td>9:50 - 10:10</td>
<td>Presentation 1</td>
<td><strong>Stem Cell-Based Technologies for Repair of Challenging Bone Defects</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Carl Gregory</strong>, Molecular &amp; Cellular Medicine</td>
</tr>
<tr>
<td>10:10 - 10:25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:25 - 10:45</td>
<td>Presentation 2</td>
<td><strong>Targeting RNA Using Mirror Image Nucleic Acids</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Jonathan Szepanski</strong>, Chemistry</td>
</tr>
<tr>
<td>10:45 - 11:05</td>
<td>Presentation 3</td>
<td><strong>Current understanding and challenges in bioprocessing of algae-derived biotherapeutics</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Zivko Nikolov</strong>, Biological &amp; Agricultural Engineering</td>
</tr>
<tr>
<td>11:05 - 11:25</td>
<td>Presentation 4</td>
<td><strong>Multiplexed gene editing and protein over-expression in plants using a Tobacco mosaic virus viral vector</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Herman Scholthof</strong>, Plant Pathology &amp; Microbiology</td>
</tr>
<tr>
<td>11:25 - 11:45</td>
<td>Presentation 5</td>
<td><strong>Engineering natural mosquito populations to prevent disease transmission</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Zach Adelman</strong>, Entomology</td>
</tr>
<tr>
<td>11:45 - 1:15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:15 - 1:35</td>
<td>Presentation 6</td>
<td><strong>Synthetic biology: A “customer’s” perspective</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Ivan Rusyn</strong>, Veterinary Medicine &amp; Biomedical Sciences</td>
</tr>
<tr>
<td>1:35 - 1:55</td>
<td>Presentation 7</td>
<td><strong>Yeast-based biosensors</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Katy Kao</strong>, Chemical Engineering</td>
</tr>
<tr>
<td>1:55 - 2:15</td>
<td>Presentation 8</td>
<td><strong>Chip-scale, label-free, real-time biomedical sensing using mid-IR integrated photonics</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Pao-Tai Lin</strong>, Electrical and Computer Engineering</td>
</tr>
<tr>
<td>2:15 - 2:35</td>
<td>Presentation 9</td>
<td><strong>DoD Advanced Regenerative Manufacturing Institute at Texas A&amp;M – Technology Road Map and Opportunities</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dr. Balakrishna Haridas</strong>, Biomedical Engineering</td>
</tr>
<tr>
<td>2:35 - 2:45</td>
<td>Closing Remarks</td>
<td></td>
</tr>
</tbody>
</table>
**KEYNOTE SPEAKER – DR. MANSOOR KHAN**

*College of Pharmacy, Texas A&M University Health Science Center*

Dr. Mansoor A. Khan has served FDA for over 11 years as the Director of Product Quality Research and a Senior Biomedical Research Scientist (SBRS) at CDER, where he helped develop regulatory policies for reviews and compliance, and led cmc review and research teams on drug delivery systems, product stability, biotech products, and biopharmaceutics. In 2015, he joined Texas A&M University as Professor and Vice Dean at Rangel College of Pharmacy in College Station, TX. Prior to joining FDA in 2004, Dr. Khan was a Professor of Pharmaceutics and Director of Graduate Program in the School of Pharmacy at Texas Tech University Health Science Center. He earned his Ph.D. degree in Industrial Pharmacy from the St. John’s University School of Pharmacy at New York in 1992. He has published over 290 peer-reviewed manuscripts, five texts including “Quality by Design for Biopharmaceutical Drug Product Development, 25 book chapters, 250 poster presentations, and more than 250 invited presentations world-wide.

Dr. Khan has held several leadership positions at the American Association of Pharmaceutical Scientists (AAPS) including elected chair of pharmaceutics and drug delivery (PDD) and the founding chair of formulations design and development (FDD). He serves on the editorial board of Pharmaceutical Technology, International Journal of Pharmaceutics, AAPSPharmsciTech, and Drug Delivery and Translational Research. As a formulations expert, Dr. Khan served as a FDA representative to EMA (European Medical Agency), WHO, USP, NIH, DoD, DARPA, NASA, and Bill and Melinda Gates Foundation. As SBRS scientist, he also served as the science policy advisor to CDER Center Director where he helped resolve complex issues of drug reviews and FDA compliance with science. He has also led the chemistry review team that approved the first 3D printed tablets in August 2015. Dr. Khan has received outstanding alumni award from St. Johns University, College of Phamacy, Excellence Award from Texas A&M University Health Science Center, over fifteen FDA/CDER Team Excellence Awards, FDA/CDER Scientific Achievement Award, and FDA/CDER Exemplary Performance Awards. Additionally he received the AAPS Research Achievement Award in Formulations Design and Development. He is an AAPS and AAiPS Fellow, and recently received the Presidential Impact Fellow honor at Texas A&M University.
1. A high-throughput and low cost impedance spectroscopy-based microsystem for precise cell position identification

N. Sobahi\textsuperscript{1}, H. Wang\textsuperscript{2}, and A. Han\textsuperscript{1,3}
\textsuperscript{1}Dept. Electrical and Computer Engineering, Texas A&M University, USA
\textsuperscript{2}Dept. Biomedical Engineering, Tsinghua University, China
\textsuperscript{3} Dept. Biomedical Engineering, Texas A&M University, USA

High-throughput single cell analysis microsystems have attracted significant attention due to its great potential and low cost in both medical and biological applications. High-speed cell/particle separation and sorting is an essential part in such microsystems, and many different methods such as flow dynamics, dielectrophoresis, and acoustophoresis have been utilized. In any of these methods it is important to identify and quantify cell/particle positions in the transverse direction in the microchannel that indicates both the degree of separation as well as how many cells have been separated. However, so far no suitable method that can detect cell/particle transverse positions at high-throughput and low-cost has been developed. Therefore here we present a high-throughput and low-cost cell/particle transverse position detection and quantification system.

Non-parallel electrodes were designed and bonded to single layer of PDMS microfluidic with tilting angles to have asymmetric electric fields due to different spacing in between. When cells/particles pass through at different positions in the transverse direction, the detected impedance peak amplitude and width (transit time) will change correspondingly. Polystyrene beads were introduced to the different transverse positions of the microchannel using a flow-focusing scheme.

Three different transverse positions were successfully detected and discriminated using the impedance peak amplitude and transit time. From top to bottom, the impedance amplitude gradually decreased while the transit time increased. The developed system has achieved high-throughput detection at 400 particle/s, and has the potential to go even higher. This microsystem holds great promise to be a powerful low-cost and high-throughput cell/particle position detection method.

Submitting author: Nebras Sobahi, Dept. Electrical and Computer Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-845-9686; E-mail: nsobahi@tamu.edu

2. A novel apple recognition method under natural light condition

W.Y. Xu\textsuperscript{12}, C.Y. Ji\textsuperscript{2}, J.A. Thomasson\textsuperscript{1}, B.X. Gu\textsuperscript{2}, B. Zhang\textsuperscript{2}, and S.J. Jiang\textsuperscript{2}
\textsuperscript{1}Dept. Biological and Agricultural Engineering, Texas A&M University, USA
\textsuperscript{2}College. Engineering, Nanjing Agricultural University, China

Real time efficiency is one of the bottleneck problems in the field of image processing, especially in the natural scene of the agricultural robot vision system. To solve the problem of the poor apple recognition performance under the natural light, two approaches of fruit color feature and distance similarity were combined to find an effective method for identifying apple fruits under natural light conditions. We built a database of 2000 apple images in almost all natural conditions. Several kinds of extreme situations were chosen: high intensity of illumination light condition, low intensity of illumination backlight condition, uneven illumination of a cloudy condition, adjacency, and severe adhesion condition. The error rate curve of the insufficient segmentation, the hit rate curve of the boundary and execution time were analyzed with the 500 apple images; the GCE, FNR, and FPR were detected with the 30 images in extreme condition. In the experimental results, it is confirmed that the GCE in the Graph-based and novel algorithm is reduced by 13% than BP algorithm, and WT algorithm in average, the GCE in new algorithm is reduced by 19% than the traditional algorithms. The hit rate of the boundary in new algorithm is increased by 21.7% and the speed is 1.83 times than Graph-based algorithm.

Submitting author: Weyue Xu. Dept. Biological and Agricultural Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-739-6481; E-mail: wyxu_1990@tamu.edu
3. A positive feedback loop involving sialidases potentiates pulmonary fibrosis

Tejas R. Karhadkar, Darrell Pilling, Nehemiah Cox, and Richard H. Gomer
Department of Biology, Texas A&M University, 301 Old Main Drive, College Station, Texas, 77843-3474

Sialic acids are often found as the distal terminal sugar on glycoconjugates such as glycoproteins, and sialidases (also called neuraminidases) remove this sialic acid. We previously found that desialylation of the plasma protein serum amyloid P (SAP) blocks its ability to regulate innate immune system cells. We examined the possibility that sialidases affect fibrosis. We used human lung sections, human cells, and the mouse bleomycin model to test the objective. In fibrotic lesions in human and mouse lungs, we found extensive desialylation of glycoconjugates, and upregulation of sialidases. TGF-β1, which is strongly associated with fibrosis, caused upregulation of sialidases in human airway epithelium cells, lung fibroblasts, and immune system cells. Conversely, addition of sialidases to human peripheral blood mononuclear cells caused these cells to accumulate extracellular TGF-β1, forming what appears to be a sialidase - TGF-β1 - sialidase positive feedback loop. Monocyte-derived cells called fibrocytes also activate fibroblasts, and sialidases potentiated fibrocyte differentiation. SAP inhibits fibrocyte differentiation, and sialidases attenuated SAP function. Injections of the sialidase inhibitors DANA and oseltamivir (Tamiflu) starting either 1 day or 10 days after bleomycin strongly attenuated pulmonary fibrosis in the mouse bleomycin model, and by breaking the feedback loop, caused a downregulation of sialidase accumulation. These results suggest that a positive feedback loop involving sialidases plays a major role in pulmonary fibrosis, and suggest that sialidase inhibitors could be useful for the treatment of fibrosis.

Submitting author: Tejas R. Karhadār, Department of Biology, Texas A&M University, 301 Old Main Drive, College Station, Texas, 77843-3474, USA; E-mail: tkarhadkar@bio.tamu.edu

4. Antimicrobial activity of a novel class of compounds against multi-drug resistant (mdr) gram-positive pathogens

K.N. Shah1,3, O. Ogün1, P.N. Shah1,3, H. Gao2, C. Hoffman1, J. Sweatt1, M. Yabe-Gill2, L. Kürti2, C.L. Cannon1,3
1Dept. Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, USA
2Dept. Chemistry, Rice University, USA
3Dept. Pediatrics, UT Southwestern Medical Center, USA

Fatal infections caused by multi-drug resistant Gram-positive pathogens are increasing at an alarming rate, yet the antimicrobial pipeline is rapidly shrinking. Resistance against several standard-of-care antimicrobials including vancomycin has been reported. MC21A (C58), a natural compound, is a strong candidate for development as a clinically relevant antimicrobial due to its ability to eradicate methicillin-resistant Staphylococcus aureus (MRSA). However, its progress has been mired by cumbersome biosynthesis and purification. We have developed a unique scheme that allows large-scale synthesis of C58 and its analogues. From a library of C58 based compounds, five non-toxic candidates, including C58, exhibit superior antimicrobial activity over standard-of-care antibiotics against bacteria growing in planktonic, stationary, and biofilm modes. These compounds inhibit and eradicate 90% of 38 test MRSA strains (MIC90, MBC90) at lower concentrations compared with vancomycin. An MRSA strain grown to stationary phase exhibits dose-dependent eradication at a higher rate with C59 treatment compared with vancomycin. Similarly, C58 treatment (16 μg/mL) results in complete eradication of established MRSA biofilms after 6h. Sequential passaging in presence of antimicrobial agents demonstrates a 256-, 28-, and a 6-fold increase in MIC upon incubation with clindamycin, linezolid, and vancomycin, respectively; MIC upon incubation with C59 remains unchanged. Additionally, these compounds are non-toxic to mammalian cells at concentrations that exert antimicrobial activity; the inhibitory concentration at median cell viability is at least three-fold higher than the MBC90. Our results demonstrate the potential of these compounds as non-toxic, next-generation antimicrobials that can be structurally optimized to yield superior efficacy over current antimicrobials.

Submitting author: Kush Shah, Dept. Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, College Station, Texas 77843, USA, Tel: +1-979-436-0867; E-mail: kshah@tamhsc.edu
5. Antimicrobial and synergistic effects of ibuprofen against resistant cystic fibrosis pathogens

1Dept. of Pediatrics, UT Southwestern Medical Center, USA
2Dept. of Internal Medicine and Microbiology, UT Southwestern Medical Center, USA
3Center for Nonlinear Dynamics and Dept. of Physics, The University of Texas at Austin, USA
4Center for Silver Therapeutics Research, Dept. of Chemistry, The University of Akron, USA
5Dept. of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center, USA
6Tashkent Pediatric Medical Institute, Uzbekistan

Several clinical trials demonstrate the benefits of ibuprofen therapy in cystic fibrosis (CF) patients, an effect that is currently attributed to the anti-inflammatory effects of ibuprofen, particularly on neutrophil recruitment. However, we have identified a direct antimicrobial effect of ibuprofen, as well. Using growth kinetics and endpoint CFU, we have demonstrated that ibuprofen reduces the growth rate and bacterial burden of several Gram-negative pathogens at concentrations ranging from 50 – 100 μg/mL. Given the relevance of P. aeruginosa biofilms in CF, we evaluated the effect of ibuprofen on biofilm growth mode PAO1 using confocal microscopy. Ibuprofen treatment (100 μg/mL) results in a growth retardation of PAO1 biofilms for up to 10h compared with untreated bacteria. Additionally, the antimicrobial activity of ibuprofen is conserved in vivo; mice infected intranasally with PAO1 (5 x 10^5 CFU) and treated with ibuprofen via gavage at 8h intervals exhibit a 92% survival compared with 57% among sham-treated mice (p = 0.0386) and ibuprofen-treated mice have lower lung and spleen bacterial burdens. Lastly, using disc diffusion and time-kill studies, we have demonstrated the ability of ibuprofen to act synergistically with several antimicrobials including silver carbene complexes (SCCs) against both Gram-negative and Gram-positive pathogens. The synergy data has led us to design a novel multifunctional molecule combining the antimicrobial activity of SCCs with the dual antimicrobial and anti-inflammatory effect of ibuprofen. Our results provide an additional explanation for the observed beneficial effects of ibuprofen and further strengthen the argument for using ibuprofen in the treatment of CF patients.

Submitting author: Parth N. Shah, Dept. of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center, College Station, Texas 77843, USA, Tel: +1-979-436-0867; E-mail: pshah@tamhsc.edu

6. A microfluidic 3D organ-on-a chip array for blood-brain barrier models

S. Jeong1, S. Kim2, J. Buonocore3, C.J. Welsh2, J. Li2 and A. Han1,3
1Dept. Biomedical Engineering, Texas A&M University, USA
2Dept. Veterinary Integrative Biosciences, Texas A&M University, USA
3Dept. Electrical and Computer Engineering, Texas A&M University, USA

Organs-on-chips are microfluidic cell culture systems created with micro manufacturing methods that include perfusion chambers inhabited by living cells in order to model physiological functions of tissues and organs. A multi-channel in vitro blood-brain barrier (BBB) platform is developed to better mimic the in vivo physiological environments and cellular responses. To investigate in cell-cell interactions, we have developed the multi-culture microsystem to accurately control the placing of multiple cell types and to form tighter tight junctions. The improved BBB-on-a chip array is enabled by utilizing more effective extracellular matrix, in vivo level of blood flow shear stress values comparable to the condition experienced by an in vivo BBB structure, as well as through the use of primary mouse endothelial cells with primary mouse astrocytes. The multi-electrode based chip array also allows this platform for higher throughput studies related to BBB against brain diseases.

Submitting author: Sehoon Jeong, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-595-7986; E-mail: sehoon.jeong@tamu.edu

7. A novel model for recellularizing natural myocardium for tissue engineering

Karis R. Tang-Quan1,2, Yutao Xi2, Doris A. Taylor1,2
As the leading cause of death in the US, cardiovascular disease and end-stage heart failure creates a vital need for healthy hearts, especially with the current shortage of available transplantable organs. One promising approach to meeting this outsize demand is decellularization and recellularization – the process of removing cells from an organ and repopulating it with new cells from the intended organ recipient. However, fully recellularizing a well-organized and functional whole heart remains a significant challenge due to the billions of cells required, low cell density, low cell-to-matrix ratio, as well as endless combinations of bioreactor parameters. Therefore, we experimented with a smaller scale recellularization model to develop an efficient methodology for recellularizing the extracellular matrix successfully. A decellularized rat heart was cut into 100 and 300 µm-thick rings and then injected with 10 million neonatal rat cardiomyocytes (NRCMs). We observed ring contractions beginning on day 3 after injection and growing stronger in culture for 25 days. Sporadic contractions and irregular rhythm were observed in most samples. Using scanning electron microscopy, whole mount confocal microscopy, and thin-section immunofluorescent staining, we located and quantified viable NRCMs at varying ring depths. Compared to the whole heart model, the present ring model with complete transmural myocardium eliminated the complications of the perfusion conditions, including flow pressure and rate; limited the impacts of vessel damage during injections; and provided a way to observe directly the interaction between cell and matrix. This model allows us to study how cardiomyocytes behave in the natural matrix, ultimately laying the foundation for future bioengineered organs.

Submitting author: Karis R. Tang-Quan, Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-310-357-1071; E-mail: karistq@tamu.edu

8. Bioorthogonal conjugation of bioactive proteins to thiol-ene click microparticles

F. Jivan1, R. Yegappan1, H. Pearce1, and D.L. Alge1,2
1Department of Biomedical Engineering, Texas A&M University
2Department of Materials Science and Engineering, Texas A&M University

Hydrogel microparticles are attractive drug delivery vehicles for tissue engineering. Covalently tethering growth factors, which typically have short half-lives, to microparticle carriers allows for sustained therapeutic presence in a localized area. However, one of the major challenges when tethering growth factors is maintaining their bioactivity. Here, we have developed a sequential click chemistry platform for poly ethylene glycol (PEG) hydrogel microparticle fabrication using thiol-ene chemistry followed by bioactive protein functionalization using biorthogonal tetræzene chemistry. Briefly, tetra-PEG-norbornene macromer and dithiothreitol, a short di-thiol linker, were mixed at a [thiol]:[norbornene] ratio of 0.75:1 to form the pre-polymer solution. Microparticles were formed using an aqueous two-phase emulsion with dextran and polymerization was achieved using UV light and lithium acrylophosphinate as the photoinitiator. Microgels demonstrated low polydispersity and the sequential reaction was monitored and confirmed via 1H NMR. Two model proteins alkaline phosphatase (ALP) and glucose oxidase (GOx) were explored to demonstrate protein tethering ability and maintenance of bioactivity. ALP and GOx conjugation to microparticles was quantified using a standard CBQCA assay. Bioactivity of ALP and GOx functionalized microparticles at varying doses were compared using an ALP or GOx kinetic assay, respectively. Free, untethered Tz-ALP and Tz-GOx were demonstrated to be equally bioactive compared to non-functionalized (NF)-ALP and GOx, respectively. Additionally, Tz-ALP and Tz-GOx functionalized microparticles showed a dose-dependent bioactivity response compared to NF-ALP and NF-GOx counterparts. On-going studies will use an electrospaying water-in-oil technique for microgel manufacturing and extend this platform to bone morphogenetic protein-2 (BMP2), which has relevance for bone tissue engineering.

Submitting author: Faraz Jivan, Dept. of Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: 979-845-6442; E-mail: fjivan1@tamu.edu

9. Bisphosphonate-functionalized poly (ethylene glycol) hydrogels for growth factor delivery

Ashley Tucker1, Faraz Jivan1, Daniel Alge1,2
1Department of Biomedical Engineering, Texas A&M University, College Station, Texas, USA
2Department of Materials Science and Engineering, Texas A&M University, College Station, Texas, USA

With over 6.3 million fractures that occur in the United States each year, usage of autogenic and allogenic bone sources are becoming dwindling treatment options. Bone Morphogenetic Protein-2 (BMP-2) has been shown to induce osteoblast differentiation, but uncontrolled release of these growth factors can cause potentially life threatening conditions. Fabrication of hydrogels as drug delivery vehicles to control release through tunability of the mesh size, rate of degradation, and chemical conjugation has been shown to mitigate these side effects. In particular, affinity ligands, such as bisphosphonate, have the ability to electrostatically interact with BMP-2 and can be tethered into hydrogel matrices to non-covalently control release and maintain the bioactivity of the growth factor. In this study, we designed and fabricated a bisphosphonate-functionalized poly(ethylene glycol) hydrogels for growth factor delivery. By characterizing the material through storage modulus and swelling ratio, we aim to design a hydrogel platform necessary for healing fractures.

**Submitting author:** Ashley Tucker, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA; Tel: +1-972-800-0367; E-mail: ashtuck589@tamu.edu

**10. Characterization of anti-microbial foams for use as prolonged field care hemostat**

K.A. Grant¹, A.D. Easley¹, S.T. Reine¹, M.B.B. Monroe¹, and D.J. Maitland¹

¹Department of Biomedical Engineering, Texas A&M University, USA

Uncontrolled hemorrhage is often cited as one of the major causes of preventable death on the battlefield. Polyurethane shape memory polymer (SMP) foams have been proposed as a potential treatment for hemorrhage due to their ability to expand to fill the space of a wound and promote blood clotting. SMP foams are a low-density, porous material that can be conditioned to transition between a primary and secondary shape. The addition of anti-microbial properties to these foams would additionally serve to improve the quality of treatment they provide. Here, we propose adding the product of an esterification reaction of a phenolic acid and triol (PAOH) to the SMP backbone to induce anti-microbial properties. A modified N, N, N', N'-Tetrakis(2-Hydroxypropyl) (HPED) monomer will be created by esterifying the PAOH with the unmodified HPED. The esterification will follow the mechanism of the Steglich Esterification reaction. The SMP foams will be synthesized using a traditional gas-blowing polyurethane fabrication technique. These foams will contain triethanolamine, hexamethylene diisocyanate, and varied amounts of unmodified and modified HPED. The foams will be characterized using anti-microbial tests in addition to thermal and mechanical analyses. The use of PAOH to modify the HPED in the synthesis of SMP foam will introduce anti-microbial properties to the foam as compared to a control foam that does not contain the modified HPED. These foams will still exhibit the high level of biocompatibility previously demonstrated by Rodriguez et al.

**Submitting author:** Katie Grant, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA; Tel: 210-601-5264; E-mail: katiegrant@tamu.edu

**11. Composite hydrogels with engineered microdomains for optical sensing at low oxygen conditions**

L.R. Bornhoeft¹, A. Biswas¹, M.J. McShane¹,²

¹Dept. Biomedical Engineering, Texas A&M University, USA
²Dept. Materials Science and Engineering, Texas A&M University, USA

There is a growing need for advanced tools that enable frequent monitoring of biomarkers for precision medicine. In this work, we present a composite hydrogel-based system providing real-time optical bioanalyte monitoring. The responsive material, alginate-in-alginate (AnAn), is comprised of an alginate hydrogel with embedded bioactive, nanofilm-coated phosphorescent microdomains; palladium tetracarboxyphenylporphyrin serves as an optical indicator, glucose oxidase or lactate oxidase as model enzymes, and layer-by-layer deposited polyelectrolyte multilayers (PEMs) as the diffusion barrier. Glutaraldehyde crosslinking of the nanofilms resulted in a dramatic reduction in glucose diffusion (179%) while oxygen transport was not significantly affected. The responses of the AnAn hydrogels to step changes of analytes at both ambient and physiological oxygen levels were evaluated, revealing controlled tuning of sensitivity and dynamic range.
Stability, assessed by alternately exposing the responsive AnA hydrogels to extremely high and zero analyte concentrations, resulted in no significant difference in the response over 20 cycles. These AnA hydrogels represent an attractive approach to biosensing based on biocompatible materials that may be used as minimally invasive, implantable devices capable of optical interrogation. The model glucose and lactate-responsive composite material studied in this work will serve as a template that can be translated for sensing additional analytes (e.g. urea, pyruvate, cholesterol) and can be used for monitoring other chronic conditions.

**Submitting author:** Bornhoeft, L.R., Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA; E-mail: lrbornhoeft@tamu.edu

**12. CRISPR/Cas9-mediated mutagenesis of an integrated transgene in the cotton genome**

Madhusudhana R. Janga¹, LeAnne M. Campbell¹, and Keerti S. Rathore¹,²,*
¹Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX, 77843-2123. ²Dept. of Soil & Crop Sciences, Texas A&M University, College Station, TX, 77843-2474.

The clustered, regularly interspaced, short palindromic repeats (CRISPR)/CRISPR associated (Cas)9 protein system has emerged as a simple and efficient tool for genome editing in eukaryotic cells. Cotton is an important crop that is grown mainly for its fiber, but its seed also serves as a useful source of edible oil and feed protein. Most of the commercially-grown cotton is tetraploid, thus making it much more difficult to target both sets of homologous alleles. Therefore, in order to understand the efficacy of the CRISPR/Cas9 system to target a gene within the genome of cotton, we made use of a transgenic cotton line previously generated in our laboratory that had a single copy of the green fluorescent protein (GFP) gene integrated into its genome. We demonstrate the use of this powerful new tool in targeted knockout of a gene residing in the cotton genome. By following the loss of GFP fluorescence, we were able to observe the cells that had undergone targeted mutations as a result of CRISPR/Cas9 activity. In addition, we provide examples of the different types of indels obtained by Cas9-mediated cleavage of the GFP gene, guided by three independent sgRNAs. The results provide useful information that will help us target native genes in the cotton plant in future.

**Submitting author:** Madhusudhana R. Janga, Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX, 77843-2123, Email: madhusudhana.janga@tamu.edu

**13. Cross-chiral RNA Aptamers: Exploring novel biochemical molecular interactions**

Adam M. Kabza¹, Jonathan T. Sczepanski¹
¹Department of Chemistry, Texas A&M University, USA

Aptamers comprised of L-RNA, the enantiomer of natural D-RNA, bind structured D-RNA molecules through tertiary interactions rather than primary sequence. This mode of recognition (termed cross-chiral recognition) has several advantages compared to Watson-Crick hybridization, including reduced off-target binding and the ability to recognize subtle changes in higher-order nucleic acids structures. L-RNA is the unnatural enantiomer of RNA, hence, L-RNA has identical physical properties to the D-enantiomer but natural enzymes and proteins are unable to recognize it and degrade it. This makes L-RNA aptamers promising nucleic acid therapeutic and diagnostic tools. Here, we attempt to increase the affinity of L-RNA aptamers to RNA targets using modified nucleobases.

**Submitting author:** Adam M. Kabza, Department of Chemistry, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-985-351-7720; E-mail: akabza@tamu.edu

**14. Design, synthesis, in silico study, and biological screening of flavin analogues as novel selective and potent antitumor agents**

Brian K. Standard¹, Waleed H. Malky², Ahmed M. Gouda³, Zakaria Y. Abd Elmageed¹, Rabaa
Al-Rousan1, Ashraf N. Abdraho2, Hamed I. Ali1*
Rangel College of Pharmacy, Health Science Center, Texas A&M University, Kingsville, Texas, United States.
2Department of Pharmacology, Faculty of Pharmacy, Umm Al-Qura University, Makkah, KSA
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Umm Al-Qura University, Makkah, KSA

Tyrosine kinases and their ATP binding sites have proven effective therapeutic targets for cancer therapy. However, many patients treated by protein kinase inhibitors, such as imatinib, can develop resistance leading to therapeutic failure. Our design applied a dual approach to enhance the polarity of our potent antitumor alloxazine nucleus by incorporation of cognate moieties into the tail of the imatinib structure with the aim of selectively interacting with the protein kinases containing glutamate residues. In vitro cytotoxic assays were conducted and 60% of the tested compounds showed IC50 < 1.0 μM against MCF-7 cells. The most active compounds were 10b, 10f, 10h, 10d, 9a, and 9e displayed IC50 values ranging from 0.04 to 0.55μM. Many compounds revealed effective IC50 values between 0.05-0.90 μM in A2780 cells. Likewise in MCF-7 cells, compound 10b revealed the most potent antiproliferative activity (IC50=0.05 μM) in comparison to other compounds. Similarly, compounds 10b, 10f, and 10h had the most potent activities against HCT116 cells with IC50 (0.05-0.17μM). Five of the most potent compounds at 10 M concentrations were screened against 20 kinases and interestingly many of these compounds revealed a notable inhibition (30-59%) of ABL1, CDK1/Cyclin A1, FAK and SRC kinases. Remarkably, 9c demonstrated 59% and 44% and 9a showed 48% and 33% inhibiton of FAK and ABL1, respectively. Compound 9b had similar inhibitory effect (42%) on FAK kinase. Annexin-V/PI apoptotic assay was performed at 0-100 M concentration range against MCF-7 cells. Compounds 10e (5 and 10μM), 10f (5 and 10μM), and 10h (1.0μM) significantly induced the early apoptotic changes of 89, 133, 100, 122, and 145%, respectively.

Submitting author: Hamed I. Ali, Dept. of Pharmaceutical Sciences, Texas A&M University, Irma Lerma Rangel College of Pharmacy, Kingsville, Texas 78363, USA, Tel: +1-361-845-9686; Email: alyismail@Pharmacy.tamhsc.edu

15. Developing novel biosensors for the “cross-chiral” detection of structured RNAs

Brian Young1 and Jonathan T. Szczepanski1
1Dept. of Chemistry, Texas A&M University, USA

Oligonucleotide biosensors, such as molecular beacons and other fluorescent hybridization probes, have become increasingly useful for the sequence specific detection of oligonucleotides through Watson-Crick hybridization. Traditional oligonucleotide biosensors have 3 notable drawbacks: 1) A tendency for off target interactions due to sequence repetition within the genome, 2) They often have difficulty melting and hybridizing to highly structured targets, 3) They are susceptible to nuclease degradation. These limitations can be mitigated through structural modifications to the probe itself. Nucleic acids composed of L- (deoxy)ribose (L-DNA and L-RNA) contain a modified backbone sugar that does not occur in nature and is a perfect mirror image of the naturally occurring D-ribose backbone. While the physical properties of L- oligonucleotides are identical to their D- counterparts, due to this chiral inversion they are invisible to cellular nucleases and are not capable of forming stable duplexes with their D-nucleic acid complement. This suggests that any interaction between D- and L- nucleic acids would occur independent of traditional base pairing. This so called “cross-chiral” interaction is proposed as an alternative recognition motif for biosensor design, with high sequence and structural specificity independent of the need to melt and hybridize to the target sequence.

Submitting author: Brian Young, Dept. of Chemistry, Texas A&M University, College Station, Texas 77843, USA; E-mail: brian.young@chem.tamu.edu


Ran Li1, Srinivas Chiguru2, Li Li2, Dongyoung Kim3, Ramraj Velmurugan1,4, David Kim3, Hong Tian5, Alan Schroit6, Ralph Mason2, Raimund J. Ober1,3 and E. Sally Ward1,7
In response to cellular stress, phosphatidylserine (PS) is exposed on the outer membrane leaflet of tumor blood vessels and cancer cells. This has motivated the development of PS-specific therapies although to date, PS-directed treatments using antibodies have met with limited clinical success. The generation of drug-conjugated PS-targeting agents represents an alternative approach, for which anti-tumor effects are critically dependent on efficient internalization and lysosomal delivery of the drug. However, the subcellular trafficking behavior of PS-specific agents and/or PS is unexplored. In the current study, we have generated PS-targeting agents by fusing PS-binding domains to a human IgG1-derived Fc fragment. The tumor localization and pharmacokinetics of several PS-specific Fc fusions have been analyzed in mice and demonstrate that Fc-Syt1, containing the Synaptotagmin I C2A domain, has superior properties in vivo. Conjugation of Fc-Syt1 to the cytotoxic drug, MMAE, results in a protein-drug conjugate (PDC) that is internalized into target cells and, due to the Ca\textsuperscript{2+}-dependence of PS binding, dissociates from PS in early endosomes. This Ca\textsuperscript{2+}-switched PDC is efficiently delivered to lysosomes and has potent anti-tumor effects in mouse xenograft models. Interestingly, although engineering Fc-Syt1 to generate a tetravalent form results in increased binding and internalization into target cells, this higher avidity variant has reduced persistence and therapeutic effects in mice compared with bivalent Fc-Syt1. Collectively, these studies demonstrate that approaches involving Ca\textsuperscript{2+}-switching and affinity tuning are of considerable value for the design of effective PS-targeting agents as well as other antibody/protein-drug conjugates platforms.

**Submitting author:** Ran Li, Department of Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, Texas 77843, USA, Tel: +1-979-436-0741; E-mail: rli@medicine.tamhsc.edu

17. **Dielectrophoresis in-droplet cell concentrator**

Song-I Han\textsuperscript{a}, Hyun Soo Kim\textsuperscript{b}, and Arum Han\textsuperscript{a}\textsuperscript{b}

\textsuperscript{a} *Department of Electrical and Computer Engineering, Texas A&M University, College Station, Texas 77843, USA*

\textsuperscript{b} *Department of Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA*

Droplet microfluidics, where water-in-oil emulsion can provide numerous independent bioreactors, has emerged as a high-throughput single-cell resolution screening tool. Although most of techniques used in conventional cell assay are realized in droplet microfluidics, adjusting cell concentration and solution exchange are still challenging parts to implement. Here, we present an in-droplet cell concentrator based on dielectrophoresis(DEP) in microfluidic technology, allowing for cell concentration adjustment as well as solution switch within droplets. Droplets encapsulating cells are produced through a standard flow-focusing droplet generator, and then flowed into the cell concentration channel that has an angled electrode pair. While droplets pass through this concentration channel, cells within a droplet are gradually focused to one side of the droplet using DEP generated from the edge of electrodes, followed by asymmetric droplet splitting using a Y-shaped junction that results in two split droplets, one containing all or most of the cells and another being empty or close to.

Effective in-droplet concentration was characterized by adjusting the applied voltage (10, 15, 20 V\textsubscript{pp}), flow rate (10, 20, 40 µl/h), and width ratio (0.15, 0.25, 0.35) of the droplet splitting microchannel with particles and *Chlamydomonas reinhardtii* strain CC-406 cells. The volume of one split droplet was reduced up to 84% compared to that of the initial droplet, which results in increasing the cell concentration by 5-fold with maximum recovery rate of 100%. Furthermore, on-chip integration of a droplet merging function after droplet splitting can provide more helpful tools by exchanging droplet solutions without any sample loss.
18. Effective computational detection of piRNAs using n-gram models and support vector machine

Chun-Chi Chen1, Wiaoning Qian1, and Byung-Jun Yoon1,*
1Dept. Electrical and Computer Engineering, Texas A&M University, USA

Piwi-interacting RNAs (piRNAs) are a new class of small non-coding RNAs that are associated with RNA silencing. The piRNAs play an important role in protecting the genome from invasive transposons in the germline. Recent studies have shown that piRNAs are linked to the genome stability and a variety of human cancers. Due to their clinical importance, there is a pressing demand for effective computational methods that could be used for computational prediction of piRNAs. However, piRNAs lack conserved structure motifs and sequence homology between different species, which makes accurate computational prediction of piRNAs challenging. In this work, we proposed a novel method, piRNAdetect, for reliable detection of piRNAs in genome sequences. The proposed method extracts effective predictive features through n-gram models, for classifying piRNAs using a support vector machine (SVM). The n-gram models are adopted to assess sequence homology to known piRNA families, which can provide helpful cues for predicting potential novel piRNAs. We demonstrate the performance of the proposed method piRNAdetect through extensive simulations based on the C. elegans and H. sapiens piRNAs in the piRBase database.

Submitting author: Chun-Chi Chen, Dept. Electrical and Computer Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-739-0875; E-mail: aky3100@tamu.edu

19. Environmental scanning electron microscopy can be used to predict insect damage to stored grain

Michael Pendleton1, Bonnie Pendleton2, and Gary Peterson1
1Micros. & Imaging Center, ILSB Bld. Rm. 1129, Texas A&M Univ., College Station, TX 77843-2257
2Agricultural Sciences, P.O. Box 60998, West Texas A&M Univ., Canyon, TX 79016-0001
3Texas A&M AgriLife Res. & Ext. Center, 1102 E. FM 1294, Lubbock, TX 79403-6603

The maize weevil, Sitophilus zeamais Motschulsky, is the most damaging insect pest of stored grain. Female maize weevils chew into sorghum, Sorghum bicolor (L.) Moench, deposit an egg, which hatches into a larva that feeds inside the kernel, pupates, and emerges as an adult. Each female weevil can produce as many as 400 eggs during her lifetime [1]. To determine resistance of grain of eight genotypes of sorghum, 5 g of grain of each genotype were put with three female and two male weevils into vials. Damage was scored (1 to 5, with 5 being most) and the vials were weighed every three weeks for 105 days. A Tescan Vega 3 XMU Environmental Scanning Electron Microscope (ESEM) was used to determine if the morphology of sorghum genotypes was related to resistance. To prepare sorghum for ESEM, a kernel of each sorghum genotype was cut in half, coated with gold using a Hummer sputter coater, and imaged in the ESEM. The degree of resistance to maize weevil was positively correlated to the distance from the pericarp to the aleurone layer for kernels of the eight sorghum genotypes. Seguifa sorghum was most resistant and had the greatest distance from the pericarp to aleurone layer. Funding provided in part by USAID and SMIL under Cooperative Agreement No. AID-OAA-A-13-00047.

Submitting author: 1Microscopy & Imaging Center, Texas A&M Univ., ILSB Bld. Rm. 1129, College Station, TX 77843-2257 USA, Tel: 979 845 1182, E-mail: mikep@tamu.edu

20. Exploring microbiomes using gnotobiotic mice

L. Yanagisawa1,3, A. Jayaraman2,3, R.C. Alaniz1,3,
1Dept. of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center
2Dept. of Chemical Engineering, Texas A&M University
3Core for Integrated Microbiota Research, Texas A&M Health Science Center

The human microbiome is composed of >100 trillion diverse, commensal microorganisms that have evolved to occupy unique niches of the human body. Collectively, these symbionts increase our genomic content by 100X and can potentially elevate our biosynthetic capacity by several orders of magnitude. Microbial community
composition and dynamics undeniably influence host health and are increasingly recognized as contributors to specific disease pathologies. As endogenous and manipulatable entities, these microbes are a promising, untapped resource for beneficial biomolecules and are novel targets for therapeutic intervention. Harnessing and maximizing the capabilities of the microbiome requires a detailed understanding of individual members, functional networks, and host-microbe interactions.

The Core for Integrated Microbiota Research (CIMR) is a new resource for TAMU investigators conducting microbiome studies. Our gnotobiotic animal facility houses a breeding colony of germ-free mice for axenic/gnotobiotic experiments. The Core provides users with germ-free mice, specialized germ-free housing, full-service animal care, and assistance in study design and implementation. The long-range goal of CIMR is to integrate gnotobiotics with on-campus expertise in metabolomics and genomics to provide Core users a seamless workflow for the complete analysis of the microbiome.

**Submitting author:** Lora Yanagisawa, Dept. of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center, Bryan, TX 77807, USA, E-mail: yanagisawa@tamhsc.edu

**21.** High-throughput bioinformatic approach for identifying receptor-like proteins in *gossypium spp.*

P. A. Jamieson¹, P. He², and S. Libo²  
¹Dept. Plant Pathology & Microbiology, Texas A&M University, USA  
²Dept. Biochemistry and Biophysics, Texas A&M University, USA

The ability of plants to mount a successful defense against pathogens hinges upon its ability to detect and respond to them. Plant resistance genes (R-genes) play a decisive role in this detection. One class of R-genes, termed Receptor-like proteins (RLPs), are implicated in a wide range of functions including both defense and development. Identifying significant RLPs in *Gossypium* (cotton) would provide breeders with critical data for developing markers for disease resistant germplasm. Canonically, RLPs contain an N-terminal signal peptide, one or more leucine-rich repeat domains, a C-terminal transmembrane helix, and often a short cytoplasmic tail. Using these characteristics, we delineate here a bioinformatic workflow whereby RLPs in various cotton species were identified.

**Submitting author:** Pierce Jamieson, Dept. Plant Pathology & Microbiology, Texas A&M University, College Station, Texas 77843, USA, Tel: 214-708-7488; E-mail: Jamieson.pierce@gmail.com

**22.** Hydrocolloid inks for solid freeform fabrication of porous hydrogel constructs

K. Gold, N. Sears¹, S. Cereceres¹, and E. Cosgriff-Hernandez¹  
¹Dept. Biomedical Engineering, Texas A&M University, USA

3D printing techniques have recently been adapted to fabricate complex tissue engineered scaffolds utilizing a wide variety of biomaterials. Control over the macro- and micro-scale geometry allows for patient-specific designs as well as precise control over scaffold properties, such as vasculature, permeability, and density, which are important for successful biomaterial constructs. Traditional hydrogels have proven difficult to print with extrusion based methods due to their low viscosity. Although stereolithography can be used to print hydrogels using curable liquids; this technique requires a large volume reservoir, post-processing, and specific geometrical constraints. To overcome such limitations, we have developed hydrocolloid inks for extrusion-based printing. This method does not require excess material or post-print curing and is capable of fabricating complex scaffolds with dimensions relevant to biomedical applications. This method emulsifies mineral oil in a hydrogel precursor solution, forming a viscous hydrocolloid ink that meets the minimum rheological properties for printing. The shear-thinning profile of these hydrocolloid inks provide a significant increase in print fidelity without the need for hydrogel additives. The inks can be extruded from the 3D printer and rapidly photopolymerized with a UV Cure-on-Dispense (CoD) adapter. The development of these hydrocolloid inks with CoD allows for 3D printed fully customizable hydrogel constructs with tunable porosity. This technology provides a platform for complex, high fidelity printing of various soft tissue engineered scaffolds.
23. In vitro evolution of L-ribonucleases capable of cleaving structured D-RNA targets

Nandini Kundu¹ and Jonathan Sczepanski¹
Dept. of Chemistry, Texas A&M University, USA

Catalytic RNAs (ribozymes) are ubiquitous in nature. A common reaction catalysed by these RNA molecules involves strand scission or cleavage of the phosphodiester bond in the RNA backbone. These enzymes could either be self-cleaving or cleave defined target RNA elements. In addition to natural RNA-cleaving ribozymes, several artificial ribozymes and DNAzymes have been evolved to cleave RNA. This activity has led to their repurposing as biomedical tools and therapeutic agents. However, traditional approaches utilise mainly Watson-Crick (WC) base pairing as the basis of enzyme-substrate recognition, where both the molecules are in the natural D-configuration. This can lead to off-target interaction with RNAs containing partial sequence complementarity. Here, we report a new class of ribonuclease ribozyme that cleaves RNA independent of hybridization. Our approach involves in vitro evolution of L-ribonucleases, which are comprised of enantiomeric L-RNA. Because of this, the L-ribozyme is incapable of forming contiguous base pairing interactions with its target and must bind through tertiary interactions, implying higher sensitivity and specificity. Given that L-RNA is completely nuclease resistant, evolution of such L-ribonucleases has potential impact in therapeutics as alternative RNA silencing agents.

Submitting author: Nandini Kundu, Dept. of Chemistry, Texas A&M University, College Station, Texas 77843, USA; email: nandunikundu@tamu.edu

24. Interconnected Cost Function Networks (iCFN): an exact algorithm for multistate protein design

Mostafa Karimi¹², Yang Shen¹²
¹Department of Electrical and Computer Engineering, Texas A&M University, USA
²TEES-Agrilife Center for Bioinformatics and Genomic Systems Engineering, Texas A&M University, USA

Computational protein design facilitates a rational and high-throughput approach to designing proteins of desired functions. It however remains a challenging problem even for a simplified single-state version where protein backbones are regarded fixed and only a single positive design objective is considered explicitly. There is a pressing demand to solve multistate protein design problems that remove the unrealistic simplifications and to find exact algorithms that guarantee optimal solutions, which would enable mechanistic insights together with experimental feedback. We have developed a novel exact algorithm called interconnected cost function networks (iCFN) to generate not only the global optimum but also the gap-free list of top solutions for multistate protein design. Our generic formulation allows for a wide array of applications for stability, affinity, and specificity design while addressing global flexibility of protein backbones and varieties of design objectives. We applied iCFN to the design of T-cell receptor (TCR) specificity for immune therapy and the prediction of estrogen receptor mutations in metastatic breast cancer. Even though these applications presented combinatorial optimization problems of unprecedented sizes to exact methods, iCFN showed drastic efficiency gains and revealed new mechanistic insights.

Submitting author: Mostafa Karimi, Dept. Electrical and Computer Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-224-5153; E-mail: mostafa_karimi@tamu.edu

25. Interfacial friction and nanomechanical properties of gossypium hirsutum l. fibers by force-distance curve-based SPM

F. Hosseinali¹, J.A. Thomasson¹, M. Elinski², and J.D. Batteas²³
¹Dept. Biological and Agricultural Engineering, Texas A&M University, USA
²Dept. Chemistry, Texas A&M University, USA
Various surface nanomechanical properties—such as elastic modulus, deformation, and adhesion—can be interpreted from SPM force-distance curves. Successive measurements of force-distance curves across a specimen surface generates maps of various local nanomechanical properties of the specimen where each pixel of a map denotes the magnitude of a given nanomechanical property of the substrate. Plant cells are covered with a thin extracellular membrane, known as the cuticle which strengthens the overall structural stability of the cell and preserves its physiological integrity. Characterization of the local nanomechanical properties of plants cuticular membranes have provided valuable insight into the understanding of plant cell growth and development (morphogenesis). The objective of this research is to measure and compare various surface attributes of Gossypium Hirsutum L. fibers from different varieties, using the SPM. Those attributes of surface which will be determined in this study include surface roughness, microscales friction, work-of-adhesion, adhesion hysteresis, and estimated contact area as obtained from the Hertz, JKR, and DMT contact mechanics models. Our preliminary results indicate that the surface characteristics of Gossypium Hirsutum L. fibers vary significantly between fiber types.

**Submitting author:** Farzad Hosseinali, Dept. Biological and Agricultural Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-777-1528; E-mail: farzad.h@tamu.edu

26. Interference-based label-free analysis of cancer cells with different metastatic potential

J. C. Contreras-Naranjo, B-H. Choi, A. Jayaraman, and V. M. Ugaz
Artie McFerrin Department of Chemical Engineering, Texas A&M University, USA

The potential use of circulating tumor cells (CTCs) for cancer diagnosis and treatment has attracted significant interest in recent years. However, CTCs are extremely rare and current technologies for CTC analysis face a number of limitations such as low recovery rates, low-throughput, and the need to identify highly specific antibodies for commonly used affinity-based capture and identification methods. Here we present a combination of microfluidics, imaging, and computational approaches aimed at the development of an innovative platform for rapid analysis of CTCs directly from whole blood. Enrichment of CTCs will be achieved using a highly selective microfluidic-based filtration architecture that operates optimally at high flow rates (mL/min range). To identify CTCs, cells will be analyzed using an interference-based label-free technique, reflection interference contrast microscopy (RICM), where interferograms embed detailed topographical information of the cell-substrate interaction, down to the nanometer-scale. Preliminary results involving PC3 and LNCaP prostate cancer cell lines with high and low metastatic potential, respectively, indicate that RICM-based analysis easily discriminates between these cell lines. These results illustrate RICM’s potential for label-free identification of highly metastatic cancer cells, a key capability in the context of CTC analysis. Therefore, RICM characterization of large populations of both blood and cancer cells will enable the collection of statistically significant libraries of information for proper identification of individual cells using machine learning algorithms. We expect that, when completed, this platform will be capable of processing 1 mL of undiluted whole blood in under 60 minutes for rapid and efficient CTC detection.

**Submitting author:** Jose C. Contreras-Naranjo, Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-979-422-1584; E-mail: clemente@tamu.edu

27. Microporous scaffolds assembled from microgel building blocks for hMSC delivery and bone tissue engineering

S. Xin\(^1\), Omar M. Wyman\(^1\) and D. L. Alge\(^1,^2\)
\(^1\)Dept. Biomedical Engineering, Texas A&M University, USA
\(^2\) Dept. Material Science and Engineering, Texas A&M University, USA

Hydrogels are widely used to deliver therapeutic cells such as human mesenchymal stem cells (hMSCs) by mimicking microenvironmental cues for tissue engineering. In conventional nanoporous hydrogels, however, cell migration and proliferation are dictated by the scaffold degradation rate, which conflicts with maintaining the designed mechanical cues to cells for tissue engineering applications. Here, we introduce a microporous hydrogel scaffold prepared by assembling electrospayed poly(ethylene glycol) (PEG) microgels to decouple these cellular processes from degradation. Microgel crosslinking and assembly both used thiol-ene chemistry in a sequential click design.
The size and stiffness of microgels were tuned by using different electrospraying parameters and molecular weights of PEG. Various bioactive peptides were also tethered in the microgels through facile thiol incorporation to tune integrin signaling. The results showed that hMSC adhesion and spreading could be successfully dictated by integrin signaling and microgel elasticity. An enhanced rate of hMSC growth was achieved within microporous scaffolds compared to conventional bulk hydrogels. Therefore, these microporous scaffolds are suitable in development of cell-constructive materials by introducing microporosity and precisely controlled microenvironmental cues.

**Submitting author:** Shangjing Xin, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-352-777-9494; E-mail: sxin@tamu.edu

### 28. Modeling LPS-induced TNF-α production in macrophages

Dongheon Lee\textsuperscript{1,2}, Yufang Ding\textsuperscript{3}, Arul Jayaraman\textsuperscript{1,3}, and Joseph Sangil Kwon\textsuperscript{1,2} (1)Artie McFerrin Department of Chemical Engineering, Texas A&M University, USA (2)Texas A&M Energy Institute, Texas A&M University, USA (3)Department of Biomedical Engineering, Texas A&M University, USA

Macrophages are ubiquitous throughout the body and indispensable in various physiological responses such as innate and adaptive immune responses, which are mediated by membrane receptors by recognizing pathogen-derived molecules. Macrophages express Toll-like receptor 4 (TLR4), and TLR4 can recognize LPS (lipopolysaccharide), which is a major component of bacterial outer membranes. Recognition of LPS initiates intracellular signaling pathways to eliminate the bacterial components. As a result, transcription factor NFκB is activated, which leads to the production of the pro-inflammatory cytokine TNF (tumor necrosis factor). TNF, in turn, initiates the TNF signaling pathway and propagates the inflammatory response. Previous studies have constructed population-level models to study the dynamics of signaling through the NFκB pathway. Predictions from the population-level model could be misleading since the model masks behaviors of individual cells. It has been shown that effects of LPS stimulation can be highly heterogeneous across the population. Therefore, we attempted to develop a stochastic model of LPS-induced NFκB signaling and TNF production in macrophages. Namely, biomolecules were modeled as states, and the corresponding kinetic parameters in the model were identified via inverse modeling. For validation, the activation of TNF and IkB in RAW macrophages under different LPS doses was quantified using flow cytometry and intracellular staining at the single-cell level. The model is expected to predict the diversity of cellular responses that cannot be captured by population-based models. Furthermore, it will be possible to simulate intercellular interactions with this stochastic model to analyze cell-to-cell communications - a step toward constructing the multicellular or tissue model.

**Submitting author:** Dongheon Lee, Dept. Chemical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-832-758-1134; E-mail: dl9@tamu.edu

### 29. Monitoring of blending process by on-line nir probe for solid dosage forms manufacturing

Sogra F. Barakh Ali\textsuperscript{1}, Sathish Dharani\textsuperscript{1}, Francesca Gay\textsuperscript{2}, Mansoor A. Khan\textsuperscript{1}, Ziyaar Rahman\textsuperscript{1}*
Rangel College of Pharmacy, Texas A&M University, College Station, Texas-77843
Artie McFerrin Dept. of Chemical Engineering, Texas A&M University, College Station, Texas- 77843

Quality-by-Design (QbD) and Process Analytical Technology (PAT) are the initiatives of the FDA to enhance the product and process understanding and built the quality form the inception of the product rather than test the quality at the end of manufacturing. PAT tools are the system of monitoring, understanding and controlling the pharmaceutical manufacturing process. The mixing process is critical in the manufacturing of solid dosage forms such as tablets and capsules. The objective of research was to understand mixing process using phenytoin as a model drug. Mixing process was performed in high-shear granulation and monitored by on-line near-infrared probe. Mixing process was monitored as a function of process parameters such as impeller speed and time. The NIR data was preprocessed by various algorithm such as multiple-scattering correction, standard-normal variate and derivatives to correct the data for baseline followed by principle component analysis. The results indicated the mixing of powder can be achieved within five minutes and PCAs analysis grouped the data in mixed and unmixed region which is correlated well with the changes in the spectrum. In summary, NIR PAT tool showed the possibility of real-time monitoring of mixing process.
30. Nanocomposite hydrogel for orthopedic tissue engineering

E. Mondragon¹, A.K. Gaharwar¹, and R. Kaunas¹,²
¹Department of Biomedical Engineering, Texas A&M University, USA
²Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, USA

Regenerative medicine has aimed to restore function to or completely replace diseased tissues. For the purpose of regenerating orthopedic tissues, human mesenchymal stem cells (hMSCs) are particularly attractive due to their capacity to differentiate into relevant cell lineages. Unfortunately, their sole use in critical-sized bone defects, which do not heal without intervention, have been disappointing due in part to poor cell retention at the injury site. Engineered tissue complexes or scaffolds have been utilized to improve cell retention, although reproducibility, poor osteoconductivity, and integration with native bone tissue have impeded the translation of cell-scaffold complexes. Thus developing engineered constructs that recapitulate the composition and structure of healthy bone tissue could address the limitations of current cell-scaffold complexes. Although collagen hydrogels have been extensively used to study cell behavior in fibrous 3D microenvironments, weak mechanical properties limit their use in bone regeneration. Due to tunable mechanical, microstructural, and bioactive properties, nanocomposite hydrogels show great promise as engineered tissues. Here we present the osteogenic bioactivity of silicate nanoplatelets (nSiO) in both monolayer and in a collagen nanocomposite hydrogel. Alkaline Phosphatase (ALP) kinetic assay confirms the addition of nSiO results in improved osteogenic differentiation of hMSCs in monolayer and in collagen nanocomposite hydrogel. In addition, rheological and cell-hydrogel contraction tests show an increase in the mechanical strength of collagen nanocomposite hydrogels due to the addition of nSiO. Taken together, the data presented here showcases the potential of nSiO-based nanocomposite hydrogels for orthopedic tissue engineering.

Submission author: Eli Mondragon, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +832 868 9624; E-mail: elmondragon26@tamu.edu

31. Osteogenically enhanced mesenchymal stem cells and their cell-derived matrices for bone tissue engineering

C. Sears¹, E. McNeill², B. Clough², S. Jaligama³, J. Kameoka³, C. A. Gregory¹,², and R. Kaunas¹,²
¹Dept. Biomedical Engineering, Texas A&M University, USA
²Dept. Molecular and Cellular Medicine, Texas A&M Health Science Center, USA
³Dept. Electrical Engineering, Texas A&M University, USA

Of the 5.6 million bone fractures that occur annually in the United States, about 10% fail to repair¹. The canonical wingless (cWnt) signaling pathway is critical for healing of bone fractures². We have demonstrated that inhibiting peroxisome proliferator-activating receptor gamma with GW9662 (GW) reduces negative cross-talk on the cWnt pathway, resulting in a pro-osteogenic human mesenchymal stem cell phenotype (OEHMSCs)³. OEHMSCs secrete an extracellular matrix (hMatrix) that mimics the composition of anabolic bone tissue and strongly enhances hMSC retention and subsequent bone repair in vivo⁴. To provide an injectable scaffold for OEHMSC delivery, we generated gelatin methacrylate (GelMA) microspheres using a custom-printed coaxial flow focusing device. The GelMA microspheres were seeded with human mesenchymal stem cells (hMSCs) and cultured in osteogenic base media containing 10μM GW for 10 days to generate constructs containing mineralized hMatrix. The hMatrix deposited onto the GelMA microspheres was demonstrated using antibodies directed against collagen types unique to hMatrix. Osteoprotegerin secretion was upregulated in GW9662-treated hMSCs. After decellularization, GelMA-hMatrix microspheres were implanted into murine critical-sized calvarial defects, followed by bone repair assessments by x-rays at 3 weeks postoperatively. We demonstrated that a composite prepared with OEHMSCs and GelMA-hMatrix microspheres has an enhanced capacity for the repair of critical-sized calvarial defects. Injectable microspheres that incorporate OEHMSCs and hMatrix will facilitate translation of this strategy to the clinic. These results together indicate that hMatrix-coating of microcarriers are effective vectors for delivery of hMSCs for bone healing.
32. Positive allosteric modulator recognition at the nicotinic acetylcholine receptor α4:α4 extracellular interface

Farah Deba1, Ze-Jun Wang1, Tasnim S. Mohamed1, David C. Chiara3, Kara Ramos1, Ayman K. Hamouda1,2.
1Department of Pharmaceutical Sciences, Texas A&M Health Sciences Center, Kingsville, TX 78363, 2Department of Neuroscience and Experimental Therapeutics, Texas A&M Health Sciences Center, Bryan, TX 77807, and 3Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Neuronal nicotinic acetylcholine receptors (nAChRs) are promising drug targets to treat several neurological disorders and nicotine addiction. There is growing evidence that positive allosteric modulators (PAMs) of nAChRs provide more favorable pharmacological specificity by binding to unique sites present only in a subpopulation of nAChRs. Furthermore, nAChR PAMs such as NS9283 and CMPI have been shown to potentiate responses of (α4)3(β2)2 but not (α4)2(β3)2 nAChR isomorph. This selective potentiation underscores the importance of the α4:α4 interface, which is present only in the (α4)3(β2)2 nAChR, as a promising drug target. In this report, using mutational analyses of amino acid residues and computational analyses we elucidated CMPI’s binding mode at the α4:α4 subunit extracellular interface and identified a unique set of amino acid residues as its affinity determinants. We found that amino acid residues α4Gly41, α4Lys64, and α4Thr66 were critical for (α4)3(β2)2 nAChR potentiation by CMPI but not by NS9283, whereas amino acid substitution at α4H116, a known determinant of NS9283 and agonist binding at the α4:α4 subunit interface, did not reduce CMPI potentiation. In contrast, mutations at α4Gln124 and α4Thr126 reduced potentiation by CMPI and NS9283, indicating a partial overlap of their binding sites. Results reported here delineate the role of amino acid residues contributing to the α4:α4 subunit extracellular interface in nAChR potentiation. They also provide structural information that will facilitate the structure-based design of novel therapeutics that target selectively the (α4)3(β2)2 nAChR.

Keywords: Positive allosteric modulators, nicotinic acetylcholine receptors, pentameric ligand-gated ion channel, drug design, CMPI, NS9283, dFBr.

33. Protein-specific process development for recovery of recombinant osteopontin from microalgae

Ayswarya Ravi1, Shengchun Guo1, Miller Tran2, Beth Rasala2, Stephen Mayfield2, and Z.Nikolov1,3
1Dept. of Biological and Agricultural Engineering, Texas A&M University, USA
2San Diego Center for Algal Biotechnology, University of California San Diego, Division of Biology, La Jolla, CA 92093, USA
3National Center for Therapeutic Manufacturing, Texas A&M University, College Station, TX 77843, USA

Microalgae such as Chlamydomonas reinhardtii is gaining prominence as a protein expression system for therapeutic proteins and other high-value products. The advantages include low-cost and simple growth media, resistance to mammalian virus contamination and absence of endotoxins. Importantly, recombinant proteins expressed in the chloroplast can be phosphorylated in vivo, preserving the functionality of the protein. The potential for recombinant protein expression and bottleneck in algal bioprocessing was investigated by expressing osteopontin (OPN), a mammalian phosphoprotein crucial to bone health. OPN, an acidic protein with 28 potential phosphorylation sites and hydroxyapatite binding motif, was expressed in the chloroplast of Chlamydomonas reinhardtii. The strategy for developing a protein purification process for OPN involves selective isolation of OPN.
from host-cell proteins. Most algal proteins are acidic and are similar to OPN in size range, RuBisCO constitutes majority of the protein fraction and is also phosphorylated. To overcome these challenges and purify OPN, the protein structure and binding mechanism to several chromatography resins including ceramic hydroxyapatite were studied. The results of this chromatography screening revealed interesting details on the properties of OPN, its size, wide range of isoelectric point (pI) and phosphorylations. Comparing the chromatography behavior of OPN from algae, to its non-phosphorylated counterpart expressed in *E. coli*, revealed the differences in OPN properties due to the capacity of the expression systems.

**Submitting author:** Ayswarya Ravi, Dept. of Biological and Agricultural Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-469-684-6665; E-mail: raviys.10h@tamu.edu

**34. Rapid fabrication of tubular scaffolds for bone regeneration via rotary jet spinning**

R.W. Reese¹, G.B.C. Cardoso², R. Kaunas¹,³
¹Dept. Biomedical Engineering, Texas A&M University, College Station, Texas, USA
²Dept. Mechanical Engineering, State University of Campinas, São Paulo, Brazil
³Dept. Molecular and Cellular Medicine, Texas A&M University, College Station, Texas, USA

Treating non-union bone defects is a major challenge in orthopedics. Through the use of rotary jet spinning (RJS [centrifugal spinning]) it has been demonstrated that it is possible to generate micro-fibrous scaffolds that possess a high degree of fiber alignment. Furthermore, RJS has a much higher production rate than similar techniques, such as electrospinning, which makes scaling up for manufacture more feasible. We have shown that by collecting these fibers on a specialized collection array it is possible to generate tubular scaffolds out of polycaprolactone or polyurethane based polymers. These scaffolds could then be used independently or in conjunction with a fracture fixation device to facilitate cellular integration and defect fixation. We hypothesize that RJS can be used to rapidly fabricate tubular scaffolds for assessment of their osteogenic potential and ability generate new bone.

**Submitting author:** Robert W. Reese, Dept. Biomedical Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: 361-446-0693; E-mail: robertreese@tamu.edu

**35. SERS based hydrogel sensors for pH and enzymatic substrates**

Y.H. You¹, A. Nagaraja², A. Biswas², H. Marks², G.L. Coté², and M.J. McShane¹,²
¹Dept. Materials Science and Engineering, Texas A&M University, USA
²Dept. Biomedical Engineering, Texas A&M University, USA

Optical glucose sensors have been studied for many years, but fully-noninvasive methods have not been successful due to weak signals (low sensitivity) and significant interferences (poor selectivity). Recent advances in materials have enabled the use of implantable chemo-optical transducers to gain selectivity and amplify signals. Most of these materials utilize organic dye-based fluorescence/phosphorescence spectroscopy, which has some long-term reliability issues related to photobleaching, sensitivity to oxygen, etc. Surface-enhanced Raman spectroscopy (SERS) systems present a potential solution for this problem, providing high sensitivity as well as stability. Previous SERS systems involved complex engineered surfaces and rigid platforms. We aimed to develop a soft, flexible material with embedded transducers to reduce failure modes. An implantable SERS sensor was demonstrated using pH-sensitive surface- enhanced Raman scattering (SERS) reporters. The nanoparticle-based pH probes were encapsulated within polyelectrolyte multilayer microcapsules, then embedded in a hydrogel matrix. These pH sensors were fully characterized for pH sensitivity and reproducibility. They were then further combined with glucose oxidase (co-encapsulated with the nanoparticles) to generate a glucose-sensitive response. The Raman signals of the system were recorded in the range of physiological glucose concentration (0 – 400 mg/dL).

**Submitting author:** Yil-Hwan You, Dept. Materials Science and Engineering, Texas A&M University, College Station, Texas 77843, USA, Tel: +1-817-404-6810; E-mail: yilhwan.you@tamu.edu
36. Sub-string/pattern matching in sub-linear time using a sparse fourier transform approach

N.T. Janakiraman, A. Vem, K.R. Narayanan, J.F. Chamberland
Dept. Electrical and Computer Engineering, Texas A&M University, USA

We consider the problem of querying a string (or, a database) of length N bits to determine all the locations where a substring (query) of length M appears either exactly or is within a Hamming distance of K from the query. We assume that sketches of the original signal can be computed offline and stored. Using the sparse Fourier transform computation based approach introduced by Pawar and Ramchandran, we show that all such matches can be determined with high probability in sub-linear time. Specifically, if the query length M = O(N^µ) and the number of matches L = O(N^λ), we show that for λ < 1 - µ all the matching positions can be determined with a probability that approaches 1 as N → ∞ for K ≤ M/6. More importantly our scheme has a worst-case computational complexity that is only O(max(N^1-µ log² N, N^µ+λ log² N)), which means we can recover all the matching positions in sub-linear time for λ < 1 - µ. This is a substantial improvement over the best known computational complexity of O((N^1-0.359µ)) for recovering one matching position by Andoni et al. Further, the number of Fourier transform coefficients that need to be computed, stored and accessed, i.e., the sketching complexity of this algorithm is only O(N^(1-µ) log N).

Several extensions of the main theme are also discussed.

**Submitting author:** Nagaraj Thenkarai Janakiraman, Dept. Electrical and Computer Engineering, Texas A&M University, College Station, Texas 77843, USA; Tel: +1-979-739-3618; E-mail: tjnagaraj@tamu.edu

37. The regeneration window: temporal dynamics of BMP2-induced middle phalanx regeneration in mice

L.A. Dawson¹, L. Yu¹, M. Yan¹, C. Dolan¹, K. Muneoka.
¹Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 USA

Successful mammalian regeneration is restricted to amputations of the distal digit tip, the terminal phalanx (P3). The regeneration response of the mouse digit tip is amputation-level specific; proximal amputations fail to elicit regeneration, instead resulting in bone truncation and scar formation. The adjacent skeletal element, the middle phalanx (P2), has emerged as a model system to investigate regenerative failure and as a site to test approaches aimed at enhancing regeneration. P2 amputation initiates dynamic tissue repair, healing via the phases of inflammation, peristeal-derived cartilaginous callus formation, woven bone callus formation, and secondary bone remodeling. We report that targeted treatment of exogenous BMP2 functions to transition the scarring event of P2 into a robust bone regeneration response mediated by the induction of a transient cartilaginous callus formed distal to the amputation plane. BMP2 initiates a regeneration response during the peristeal-derived cartilaginous healing phase of P2 bone repair, yet fails to induce regeneration in the absence of peristeal tissue, or after the peristeal-cartilage has been remodeling into a boney callus. These findings provide evidence that a temporal component associated with wound maturation exists in P2 induced-regeneration, termed the ‘regeneration window’, whereby cells are transiently responsive to signals after amputation. Simple re-injury of the previously amputated (i.e. scarred) P2 stump acts to reinitiate the endogenous bone regeneration attempt, complete with peristeal chondrogenesis and therefore re-opening of the ‘regeneration window’, thus recreating a regeneration-permissive wound environment in which exogenous BMP2 functions to induce regeneration of the previously scarred P2 stump.

**Submitting author:** Lindsay A. Dawson, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843 USA. Phone: 979-845-1730. Email: ldawson@cvm.tamu.edu

38. The role of exosomes-associated microRNAs in developing drug resistance in melanoma

Shaimaa A. Gad¹, Hamdy EA Ali¹, Hamed Ismail-Aly¹, Zakaria Y. Abd Elmageed¹
Department of Pharmaceutical Sciences, Rangel College of Pharmacy, Texas A&M Health Science Center, Kingsville, TX 78363
BRAFV600E mutations have been reported in up to 70% of cutaneous melanoma and proposed as a major contributor to its oncogenesis and is associated with poor prognosis. Selective BRAFV600E inhibitors, such as Vemurafenib, have now been established as a standard therapy for metastatic melanomas with BRAFV600E mutation. Although the successful targeting of BRAFV600E in these patients improves survival, resistance to this therapy emerges rapidly. Hence, identifying the molecular mechanisms by which melanoma cells with BRAFV600E mutations develop resistance to such therapy is urgently needed. We aim to determine the role of exosomes in developing Vemurafenib resistance possibly through biological materials transferred in their vesicular cargo.

Methods: In this study, we utilized two Vemurafenib resistant melanoma cell lines, WM9838BR and A375-NRASQ61K in addition to their parental cells. We isolated exosomes from the conditioned media of these cells and performed microRNA (miR) and mRNA microarray analyses. Real-Time PCR and Western blot were used as tools to validate these miRs and their target genes.

Results: Our results demonstrate that profiling resistant cells have differential expressions of miRs and transcripts in response to acquiring drug resistant. The upregulated miRs were miR-302d and miR-590-5p (3.6 and 1.7 fold change) whereas the downregulated miRs were miR-4279, miR-149, miR-625 (12.6, 8.6, and 7.2 fold change, respectively). One of the miR-target genes was ABCF2 and RAB40A. Our ongoing study designed to determine their contribution to drug resistance on the level of cells and human samples.

Conclusions: Our findings highlighting the role of exosomes-associated miRs in developing drug resistance of melanoma patients who had BRAFV600E. These miRs can be used as markers to re-stratify the patients based who are more likely to develop resistance. Further studies are warranted to investigate the role of exosomes-cargo in developing drug resistance.

39. Viral delivery of a gene editing tool for transient screening of gene function

Will B. Cody¹, Herman B. Scholtz¹, and T. Erik Mirkov¹,²
¹ Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX
² Texas A&M AgriLife Research Center, Weslaco, TX

The CRISPR/Cas9 gene editing platform has been adapted as a transient screening device in biological model systems, but there are currently limited tools available for plant pathologists. Here, we used the Tobacco mosaic virus viral vector, TRBO, to deliver biologically active single guide RNA (sgRNA) constructs in Nicotiana benthamiana by measuring the presence of genomic indels (inserts and deletions) when co-delivered with Cas9 through agroinfiltration. Indel percentages averaged ~70% within 7 days post-inoculation (dpi) when targeting the mgfp5 coding region of GFP-expressing N. benthamiana 16c plants. High editing efficiencies translated to a knockdown in GFP production and green fluorescence in 16c plants leaves. The N. benthamiana paralogs Argonaute 1-H and Argonaute 1-L were targeted using one sgRNA construct, which created indels within both of the native genes. Similar indel efficiencies were observed when NbAGO1 and mgfp5 sgRNAs were co-delivered (multiplexed) using a single TRBO delivery construct. Additionally, we used TRBO to deliver an RNA transcript with a sgRNA adjoining a GFP protein coding region, which successfully created both indels in the genomic mgfp5 target and viral based protein overexpression. These results show that TRBO-sgRNA-Cas9 co-infiltration provides a transient gene knockout system that can be used for functional genetic studies.

Submitting author: Will B. Cody, Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843, USA; E-mail: willbcody@tamu.edu