2019 McGowan Retreat
Poster Abstracts

Cellular and Gene Therapy

1. **Abigail Allen**, Ryan Martin, Walter Storkus, Michael Lotze and Partha Roy. *A Therapeutic Role for Profilin 1 in the Progression and Metastasis of Renal Cell Carcinoma*

2. **Lawrence P. Andrews**, Sasikanth Manne, E. John Wherry, Creg J. Workman and Dario A.A. Vignali. *Synergistic interactions between PD1 and LAG3 limit anti-tumor immunity*

3. **Oliver Beale** and Deniz Dalkara. *Utilization of Superparamagnetic Nanoparticles and Columns for One-Step Purification of Recombinant Adeno-Associated Viral (AAV) Vectors*

4. **Michael R. Behrens**, Felicia Y. Scott and Warren C. Ruder. *Biomagnetic Genetically Programmed Multicellular Microrobots*

5. **Zachary Clemens**, Abish Pius, Amrita Sahu, Sunita Shinde, Aaron Barchowsky and Fabrisia Ambrosio *Administration of alpha-Klotho systemically enhances skeletal muscle regeneration*

6. **Maria Cohen**, Lukas Schimunek, Rami A. Namas, Haley Lindberg, Fayten El-Dehaibi, A. Murat Kaynar, Timothy, R. Billiar and Yoram Vodovotz. *Sevoflurane is Associated with Worse Clinical Outcomes and Altered Inflammatory Markers in Blunt Trauma Patients: Potential Role of Single Nucleotide Polymorphisms rs4715332 and rs1695*

7. **Dan Crompton**, David Chan, John Waters and Marina V. Kameneva *In vitro optimization of hemoglobin solution for use in sickle cell hemoglobin replacement therapy*

8. **Evan Delgado**, Junyan Tao, Madeleine Leek, Satdarshan Monga and Andrew Duncan. *Understanding the role of the pleiotropic scaffolding protein IQGAP1 in Hepatocellular Carcinogenesis*

9. **Asim Ejaz**, Michael W. Epperly, Joel S. Greenberger and Peter J. Rubin. *Molecular basis of Adipose-Derived Stem Cell (ASC) therapy for management of Radiation Induced Fibrosis (RIF)*

10. **Chigozirim Ekeke**, Michael Lotze and Rajeev Dhupar. *Making Cold Malignant Pleural Effusions Hot: Development of a Murine Model of MPE*

11. **David Gau**, Souvik Chakraborty, David Boone, Alan Wells and Partha Roy. *MRTF/Profilin is an important signaling axis for metastatic outgrowth of triple negative breast cancer cells*

12. **David Gau**, Lucile Vignaud, Jordan Strum, Paul Francoeur, David Koes, Xavier Guillonneau and Partha Roy. *First-generation small molecule antagonists of profilin1 suppresses pathological retinal neovascularization*

13. **Lauren V. Huckaby**, Marie Billaud, Jennifer Hill, Tara Richards, Julie Phillippi and Thomas G. Gleason. *Sex Differences in Oxidative Stress Response in Bicuspid Aortic Valve Aortopathy*

14. **Noriyuki Kashiyama**, Robert L. Kormos, Yasumoto Matsumura, Shin-ichi Higuchi, Lindemberg M. Silveira-Filho, Garrett Coyan, Jiang Hong Bin, Antonio D'Amore and William R. Wagner. *Adipose Derived Stem Cells Enhance Cardiac Function Preservation of a Biodegradable Cardiac Patch and Increase Vascularization in Rats with Subacute Myocardial Infarction*

15. **Karis Kosar** and Kari Nejak-Bowen. *Treatment of a Mouse Model of Cholestasis with a Thyromimetic Improves Biliary Injury But Exacerbates Hepatocyte Injury*

16. **N. G. Lamson**, R. Ball, K. Suri, S. Xian, A. Zhang, V. Ahuja, A. Berger and K. A. Whitehead. *Strawberry Polyphenols are Intestinal Permeation Enhancers for Oral Drug Delivery*

17. **Madeleine P. Leek**, Evan R. Delgado, Patrick D. Wilkinson, Frances Alencastro, Narita Roy, Kero Kamel and Andrew W. Duncan. *The Role of Diploid Hepatocytes in Promoting Regeneration Following Acetaminophen Toxicity*

18. **Jingjing Li**, Wensheng Zhang, Chiaki Komatsu, Yong Wang, Moriah Johngrass, Kia Washington, Kacey Marra, Peter J Rubin, Vijay Gorantla, Lauren Kokai and Mario Solari. *Local Delivery of Adipose-Derived Mesenchymal Stem Cells Promotes Immunomodulation and Allograft Survival in Vascularized Composite Allotransplantation (VCA)*

19. **Jr-Jiun Liou**, Shenghuo Tian, Michael Yee, Paul Kinchington and Jonathan P. Vande Geest. *Manipulating Gene Expression of Human Lamina Cribrosa Cells and Astrocytes*

20. **Jason Lohmueller**, Adam Butchy, Yaniv Tivon, Natasa Miskov-Zivanov, Alexander Deiters and Olivera Finn. *Engineering universal CAR and SynNotch receptors for programmable antigen targeting*

21. **Bo Ma**, Kyle Sylakowski, Yuhan Jiang, Hanshuang Shao and Alan Wells. *Bi-directional and diametrical regulation of mesenchymal stem cells and epithelial-mesenchymal plasticity in prostate cancers*

Presenter is in **bold**

Monday, March 11, 2019 5:00-6:00 pm (odd numbers), 6:00-7:00 pm (even numbers)
Tuesday, March 12, 2019 5:00-6:00 pm (all numbers)

22. **Hikaru Mamiya**, Amrita Sahu, Amin Cheikhi, Sunita Shinde, Samuel Luketich, Gabriele Nasello, Bennette Van Houten, Antonio D'Amore, Aaron Barchowsky and Fabrisia Ambrosio. *Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype*

23. **Brian Martin**, Beth Gabris, Xuwen Wang, Guillermo Romero and Guy Salama. *Cardioprotective Actions of Relaxin via Wnt Signaling*

24. **Meghan Mooring** and Dean Yimlamai. *Using CyTOF to elucidate the signals controlling progenitor cells during liver regeneration*

25. **Abish Pius**, Amrita Sahu, Zachary Clemens, Sunita Shinde, Aaron Barchowsky and Fabrisia Ambrosio. *AAV delivery of α-Klotho: Gene therapy as a strategy to counteract sarcopenia*

26. **C. Reyes**, L. Mo, D. Guimaraes, A. Braganza, K. Quesnelle, Y. Wang and S. Shiva. *Nitrite Regulates Mitochondrial Dynamics to Inhibit Vascular Smooth Muscle Cell Proliferation*

27. **Dayana B. Rivadeneira**, Kristin DePeaux, Tracy Tabib, Ashley V. Menk, Padmavathi Sampath, Robert Lafyatis, Saumendra N. Sarkar, Stephen H. Thorne and Greg M. Delgoffe. *Oncolytic virus immunotherapy-induced remodeling of antitumor immunity is improved through vector-encoded metabolic modulation*

28. **Jacquelyn Russell**, Hirohisa Okabe, Sucha Singh, Minakshi Poddar, Marc Abrams, Kari Nejak-Bowen and Satdarshan Monga. *Lack of Beta-catenin in Hepatocytes Impairs Proliferation and Promotes Liver Progenitor Cell-Mediated Repair in Response to Hepatic Injury*

29. **Nairita Roy**, Patrick D. Wilkinson, Evan R. Delgado, Frances Alencastro, Madeleine P. Leek, Michael J. Reynolds, Sruti Shiva and Andrew W. Duncan. *Role of SLC25A34, an uncharacterized mitochondrial protein, in fatty acid metabolism and mitochondrial respiration in primary hepatocytes*

30. **Amrita Sahu**, Hikaru Mamiya, Sunita Shinde, Amin Cheikhi, Lia Winter, Nam Vo, Donna Stolz, Vera Roginskaya, Wan-yee Tang, Claudette St. Croix, Laurie Sanders, Michael Franti, Ben Van Houten, Thomas Rando, Aaron Barchowsky and Fabrisia Ambrosio. *Age-related declines in α-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration*

31. **Aaron Gabriel Sandoval**, Jason Brant and Malcolm Maden. *African spiny mouse (Acomys) regeneration following acute, chronic, and volumetric muscle loss injuries*

32. **Ashwin Somasundaram**, Anthony R. Cillo, Lauren Oliveri, Maria Velez, Sona Joyce, James G. Herman, Katie S. Nason, John M. Kirkwood, Robert L. Ferris, Tullia C. Bruno and Dario A. A. Vignali. *IL-6 and IL-8 drive IR-specific immune suppression of effector, memory and naïve, peripheral blood CD8+ T cells in cancer patients*

33. **Hengyun Sun**, Jingjing Li, Wensheng Zhang, Chiaki Komatsu, Yong Wang, Moriah V. Johngrass, Kia Washington, Kacey Marra, Peter J. Rubin, Vijay Gorantla, Lauren Kokai and Mario Solari. *Local Delivery of Adipose-Derived Stem Cells Promotes Allograft Survival and Durable Tolerance in Vascularized Composite Allotransplantation*

34. **Kyle Sylakowski**, Amritha Justin and Alan Wells. *The Angiogenic Capability of Mesenchymal Stem Cells Coupled with Tenascin-C Under Hypoxic Conditions*

35. **Kien Tran**, Mark Murdock, Stephen Badylak and Kyle Orwig. *Effects of Matrix-Bound Nanovesicles in Human Spermatogonial Stem Cell Culture*

36. **Daniel B. Whitefield**, Li Lan, Shelly Peyton and Kris Noel Dahl. *Mechanical Response of Chromatin to DNA Damage*

37. **Patrick D. Wilkinson**, Evan R. Delgado, Frances Alencastro, Madeleine P. Leek, Nairita Roy, Matthew P. Weirich, Elizabeth C. Stahl, P. Anthony Otero, Maelee I. Chen, Whitney K. Brown, Michael Oertel and Andrew W. Duncan. *Polyploidy in Liver Regeneration and Adaptation to Chronic Injury*

38. **Fatih Zor**, Huseyin Karagoz, Lu Liu, Vijay S. Gorantla and Jelena M. Janjic. *New theranostic approaches to chronic sterile inflammation and immune rejection monitoring and treatment*

39. **Daniel A. Zuppo**, Maria A. Missinato and Michael Tsang. *Foxm1 drives Cardiomyocyte Proliferation during Zebrafish Cardiac Regeneration*

Computation and Modeling

40. **Ali Mubin Aral**, Ruben Zamora, Derek Barclay, Jinling, Yin, Fayten El-Dehaibi, Vijay Gorantla and Yoram Vodovotz. *Elucidation and Integration of Tissue-Specific, Protein-Level Inflammatory Networks following Vascularized Composite Allotransplantation*

41. **Shaniel Bowen**, Pamela Moalli and Steven Abramowitch. *Defining and Comparing Mechanisms of Uterovaginal Prolapse Repair Failure*

42. **Leonid Emerel**, James Thunes, Trevor Kickliter, Marie Billaud, Julie A. Phillippi, David A. Vorp, Spandan Maiti and Thomas G. Gleason. *Pre-Dissection-Derived Geometric and Distensibility Indices Reveal Increased Peak Longitudinal Stress and Stiffness in Patients Sustaining Acute Type A Aortic Dissection: Implications for Predicting Dissection*

43. **Tian Yong Foong**, Yi Hua, Alexandra Gogola and Ian A. Sigal. *Modeling collagen fiber recruitment across the corneoscleral shell*

44. **Ronald Fortunato**, Chao Sang, Anne Robertson and Spandan Maiti. *Failure Biomechanics of Arterial Tissue*

45. **George C. Gabriel**, Nathan Salamacha, William T. Reynolds, Tuantuan Tan, Xiaoqin Liu, Hisato Yagi, Abha Bais, Ashok Panigrahy, Dennis Simon, Yijen Wu and Cecilia Lo. *Characterization of Neurodevelopmental Defects Associated with a Mouse Model of Hypoplastic Left Heart Syndrome*

46. **Eric Lambert*, Michele Herneisey***, Lu Liu, James K. Drennen, Jelena M. Janjic (*Equal contribution). *Quality by Design: methodology to boost process understanding in nanomedicine*

47. **Eric Lei**, Kyle Miller, Michael Pinsky and Artur Dubrawski. *Characterization of Multi-View Hemodynamic Data by Learning Mixtures of Multi-Output Regressors*

48. **Xinyu Li**, Michael R. Pinsky, Gilles Clermont and Artur Dubrawski. *Leveraging Routine Blood Draws to Predict Risk of Hemorrhagic Shock Before Surgery*

49. **Ngoc B. Pham**, Wen Liu, Nathan R. Schueller, Ellen S. Gawalt, Yong Fan and Wilson S. Meng. *Kinetic Model Simulation and Antigenicity Analysis of a Miniaturized Fc-Binding Domain for Local Deposition of Antibodies*

50. **Feng Shan**, Anthony R Cillo, Tullia C Bruno, Panayiotis V. Benos and Dario A.A. Vignali. *Defining transcriptional regulation of CD8+ T cells in HPV(+) and HPV(-) head and neck cancer via gene regulatory networks*

51. **Elaine Soohoo**, Lewis K. Waldman and Dennis R. Trumble. *Computational Assessment of Cardiac Hemodynamics and Biomechanics for a Torsional Ventricular Assist Device (tVAD)*

52. **Anthony Wertz**, Michael R. Pinsky, Gilles Clermont and Artur Dubrawski. *Granularity and Parsimony of Hemodynamic Vital Signs Data Impact Accuracy and Timeliness of Assessment of Physiologic State*

53. **Joo Heung Yoon**, Yang Chen, Michael Pinsky and Gilles Clermont. *Non-invasive Hemorrhage Detection Approach using Photoplethysmography*

Medical Devices

54. **Edgar Aranda-Michel**, Lewis K. Waldman and Dennis Trumble. *Beating Heart Simulation of Left Ventricular Compression for Heart Failure*

55. **Jennifer M. Armen**, Ngoc Pham, Wilson S. Meng and Ellen S. Gawalt. *Analysis of the cross-linked ionic peptide, EAK16-II*

56. **Ernesto Bedoy**, Mike Urbin and Douglas Weber. *A High-Density Electrode Array for Mapping Corticospinal Muscle Recruitment After Stroke*

57. **Garrett Coyan**, Lindemberg Silveira Filho, Yasumoto Matsumura, Samuel Luketich, William Katz, Vinay Badhwar, William Wagner and Antonio D'Amore. *Acute In Vivo Functional Assessment of a Biodegradable Stentless Elastomeric Tricuspid Valve*

58. **Eoghan M. Cunnane**, Niall F. Davis, Alan J. Ryan, Jochen Hess, Justin S. Weinbaum, Michael T. Walsh, Fergal J. O'Brien and David A. Vorp. *Improving urinary catheter safety and tissue engineered urethral scaffolds though an enhanced understanding of human urethral biomechanics*

59. **Vishaal Dhamotharan**, Ryan Orizondo and William Federspiel. *In-vitro flow characterization of PAAL & P-PAL using dimensional analysis for detection of abnormal flow conditions*

60. **Leah Dickey**, Lu Liu, Shannon Loftus, Michele Herneisey and Jelena M. Janjic. *Quality Assessment and Optimization of Nanoemulgels for Local Inflammation Treatment*

61. **Moataz Elsisy**, Bryan Tillman, Catherine Go and Youngjae Chun. *Design and Manufacturing A Novel Customizable Nitinol-PTFE Stent Graft for Effective Torso Hemorrhage Control*

62. **Firuz Feturi**, Joshua M. Barnett, Bo Xiao, Yolandi van der Merwe, Xinzhu Gu, Evan Katzel, Mario Solari, Raman Venkataramanan, William Wagner, Daniel Simons, Michael B. Steketee and Kia M. Washington. *Local Delivery of FK506 with Impregnated Nerve Wraps Accelerates Nerve Regeneration in Infraorbital Nerve Transection and Repair Model*

63. **Catherine Go**, Jenna Kuhn, Moataz Elsisy, Youngjae Chun and Bryan Tillman. *A Novel Retrievable Rescue Stent as a Comprehensive Solution to Non-Compressible Traumatic Hemorrhage*

64. **Aimon Iftikhar**, Alexis Nolfi, Branimir Popovic and Bryan Brown. *Development of a Novel Rabbit Surgical Model of Pelvic Reconstruction for the Use of Testing an IL-4 Eluting Coated Polypropylene Mesh*

65. **Jenna Kuhn**, Catherine Go, Moataz Elsisy, Yanfei Chen, Youngjae Chun and Bryan Tillman. *An E-Mag Cannulation Approach Reduces Radiation Exposure During Simulated Fenestrated Endograft Repair*

66. **Tell Lovelace**, Alexander Rupprecht, Rachelle Palchesko-Simko, Jared Romeo, Wilson Meng and Ellen Gawalt. *Immobilization of Anti-Platelet Molecules on Implant Materials*

67. **Alexandra G. May**, Ryan A. Orizondo, Brian J. Frankowski, Peter D. Wearden and William J. Federspiel. *Acute In Vivo Performance of a Pediatric Ambulatory Artificial Lung*

68. **Alexandra G. May**, Ryan A. Orizondo, Brian J. Frankowski, Ergin Kocyildirim, Jonathan D'Cunha and William J. Federspiel. *7-day In Vivo Performance of a Low-Flow Extracorporeal CO2 Removal Device*

69. **Alexis Nolfi**, Vishal Jhanji, Mangesh Kulkarni and Bryan Brown. *Polyelectrolyte multilayer coating for delivery of IL-4 from contact lenses for dry eye disease*

70. **Katelin S. Omecinski**, Brian J. Frankowski and William J. Federspiel. *Characterization of Floating Impeller Phenomena in an Integrated HFM bundle and Centrifugal Pump Design*

71. **Drake Pedersen**, Antonio D'Amore and William R. Wagner. *Characterizing Deformation in Tissue Engineered Heart Valves under Dynamic Loading Conditions*

72. **Shivbaskar Rajesh**, Daniel Crompton, James F. Antaki and Marina V. Kameneva. *Effect of turbulent flow on damage to blood cells using the in vitro model of the assisted blood circulation*

73. **Constance Robbins**, Jason Yang, James F. Antaki and Jana M. Kainerstorfer. *Hand-held optical imaging for breast cancer therapy prediction*

74. **Nicholas L. Robbins**, Matthew J. Wordsworth, Michael R. Sippel, Jennifer M. Cox, Zachary T. Homas, Bijaya K. Parida, Margaux S. Salas, Vijay S. Gorantla, Warren C. Breidenbach, George E. Wolf, Col Michael R Davis and Col Erik K. Weitzel. *Prevention of Ischemia-Reperfusion Injury and Chronic Rejection in a Porcine Vascularized Composite Allotransplantation Model*

75. **Kaylene Stocking**, Alberto Vazquez and Takashi Kozai. *Intracortical neural stimulation with untethered, ultrasmall carbon fiber electrodes mediated by the photoelectric effect*

76. **Mallory R. Wampler**, Lu Liu, Michele L. Herneisey, Margaux M. Salas, Jennifer Cox-Hinshaw, Shannon Loftus, Vijay S. Gorantla, Jelena M. Janjic and Erik K. Weitzel. *Theranostic Analgesic Regenerative Gel-Emulsion Technology (T.A.R.G.E.T.) Platform for Local Analgesia and Promotion of Nerve Regeneration*

77. **Gary Yu**, Filip Istvanic, Xucai Chen, Mehdi Nouarie and John Pacella. *Ultrasound-targeted microbubble cavitation with sodium nitrite synergistically enhances nitric oxide production and microvascular perfusion*

Tissue Engineering

78. **Arianna Adamo**, Giovanni Spiaggia, Garrett Coyan, William R. Wagner and Antonio D'Amore. *Bioengineered the Cordae Tendineae apparatus*

79. **Reem Azar**, Harmanvir Ghuman, Stephen Badylak and Michel Modo. *Mechanisms of extracellular matrix (ECM) hydrogel biodegradation: an in vitro assay*

80. **Ali Behrangzade**, Jr-Jiun Jean Liou, Ehab Tamimi, Catalina Ardila, David Harris, Tom Doetschman, Marie Billaud, William Wagner and Jonathan Vande Geest. *Design and Characterization of a Compliance-matched Biopolymer Tissue-Engineered Vascular Graft*

81. **Andrew Bradshaw**, Jelena Grahovac, Amanda Clark, Linda Griffith and Alan Wells. *The Therapeutically Induced Matrisome (Tim) Protects and Promotes Metastatic Disease*

82. **Bryn L Brazile**, Bin Yang, Andrew Voorhees and Ian A Sigal. *Visualizing the microvasculature of the optic nerve head and their changes during intraocular pressure increases*

83. **Michael J. Buckenmeyer**, Ziyu Xian, Srujan Dadi, Aimon Iftikhar, Alexis L. Nolfi, Meena Sukhwani, Kyle E. Orwig and Bryan N. Brown. *The Development of Ovarian Hydrogels as an Alternative Strategy for Fertility Preservation*

84. **Adam Chin**, Jingming Chen, Tyler Swenson, Juan Taboas and Alejandro Almarza. *In Vitro Differentiation of BMSCs in PGH and Gelatin Hydrogels*

85. **Madeline Cramer**, Jenna Dziki, George Hussey, Heth Turnquist and Stephen Badylak. *MBV-associated IL-33 Protects Against Chronic Heart Transplant Rejection*

86. **Anjani Ravindra**, William D'Angelo, Li Zhang, Janet Reing and Stephen Badylak. *Growth & differentiation of human bronchial epithelial cells on decellularized trachea ECM scaffold for airway replacement*

87. **Megan DeBari**, Rachel Niu, Bin He and Rosalyn Abbott. *Controlled Silk Degradation Using Non-Invasive Ultrasound for Tissue Regeneration*

88. **Bryant Fisher**, Jennifer C. Hill, Marie Billaud, Tara D. Richards, Thomas G. Gleason and Julie A. Phillippi. *Adventitial Extracellular Matrix Hydrogel Improves Sprouting of Human Thoracic Aortic Aneurysm-Derived Pericytes*

89. **Kenneth J. Furdella**, Shinichi Higuchi, Kang Kim, William R. Wagner and Jonathan P. Vande Geest. *Compliance Manipulation of Polycaprolactone/Gelatin Tissue Engineered Vascular Grafts in a Rat Model*

90. **Martin Haschak** and Bryan Brown. *Age-related compositional and biomechanical changes in the cardiac extracellular matrix promote altered macrophage phenotype and function*

91. **Shinichi Higuchi**, Antonio D'Amore and William R. Wagner. *Creating innervated vascularized muscle flaps from elastic, cellularized biocomposites developed in situ for facial muscle reconstruction*

92. **Bistra Lordanova**, William Klunk and Alberto Vazquez. *Hypercapnia exposes deficiencies in cerebrovascular response and tissue oxygenation of transgenic AD mice*

93. **Irona Khandaker**, Moira Geary, Martha Funderburgh and James Funderburgh. *A Novel Mouse Model for Corneal Scarring*

94. **Biao Kuang**, Yuwei Liu, Rocky S. Tuan and Hang Lin. *Robust Bone Formation through the Developmental Condensation and Endochondral Ossification of human Mesenchymal Stem Cells within their Own Extracellular Matrix*

95. **Yoojin Lee**, Urszula Zdanowicz, George Hussey and Stephen F. Badylak. *Matrix-bound Nanovesicles for Treatment of Achilles Tendinopathy*

96. **Zhong Li**, Zixuan Lin, Monica R. Lopez, Benjamen O'Donnell, Xinyu Li, Ian J. Moran, Peter G. Alexander, Stuart B. Goodman, Bruce A. Bunnell, Hang Lin and Rocky S. Tuan. *Organ-on-a-chip System for the Modeling of Synovial Joint Pathologies*

97. **Zixuan Lin**, Zhong Li, He Shen, Xinyu Li, Rocky S Tuan and Hang Lin. *iPSCs-Derived Osteochondral Tissue Chip to Model Joint Physiology and Osteoarthritis Pathology*

98. **Jr-Jiun Liou**, Catalina Ardila, Kenneth Furdella, Ali Behrangzade and Jonathan P. Vande Geest. *Cord Blood-Derived Endothelialization of Tissue-Engineered Vascular Grafts*

99. **Samuel K. Luketich**, Garrett Coyan, Lindemberg M. Silveira-Filho, Yasumoto Matsumura, Drake Pedersen, Arianna Adamo, Casey C. Tompkins-Rhoades, Salvatore Pasta, William R. Wagner and Antonio D'Amore. *Development and assessment of a novel tissue engineered mitral valve with an engineered chordal apparatus*

100. **Katherine Lorentz**, Jeffrey Krawiec, Darren Haskett, Justin Weinbaum, Morgan Fedorchak, Antonio D'Amore, William R. Wagner, Steven Little and David Vorp. *Cytokine Mimicking Microspheres for Use in Porous Scaffolds*

101. **Tyler Meder**, Travis Prest, Lucile Marchal, Chloe Kaunitz, Clint Skillen and Bryan Brown. *Combining a Peripheral Nerve Matrix Derived Hydrogel and Post-Surgical Therapy for Improving Functional Recovery Following Nerve Reconstruction*"

102. **Wai Hoe Ng**, Elizabeth Johnston, Jun Jie Tan and Xi Ren. *Simultaneous Heart and Lung Co-differentiation by Modulating WNT, Activin A and BMP4*

103. **Kevin Pietz**, Connor Wiegand and Ipsita Banerjee. *Differentiation of hPSCs into Islet-mimetic cells: Encapsulation versus Suspension Culturing*

104. **Alessandro Pirosa**, Karen Clark, Hang Lin, Mateus Pinho, Yuanheng Yang, Rocky S. Tuan and Peter G. Alexander. *Development of human organotypic culture models for teratogenesis assessment on limb development*

105. **Sahil K Rastogi**, Jacqueline Bliley, Daniel Shiwarski, Raghav Garg, Adam Feinberg and Tzahi Cohen-Karni. *Novel Three-Dimensional Fuzzy Graphene (3DFG)-Based Ultra Microelectrodes Array for Sub-Cellular Electrical Recordings*

106. **Benjamin K. Schilling**, M. Asher Schusterman II, Deok-Yeol Kim, Alex Repko, Katarina Klett, George J. Christ and Kacey G. Marra. *Adipose-Derived Stem Cells Partially Mitigate Muscle Atrophy after Peripheral Nerve Injury in the Rodent Model*

107. Hikaru Mamiya, **Sruthi Sivakumar**, Amrita Sahu, Amin Cheikhi, Sunita Shinde, Adam Wise, Samuel Luketich, Gabriele Nasello, Philip Leduc, Bennett Van Houten, Antonio D'Amore, Aaron Barchowsky and Fabrisia Ambrosio Establishing the role of ECM stiffness in skeletal muscle regeneration

108. **Ning Wang**, Yuzhao Huang, Rocky S. Tuan and Hang Lin. *Enhancing Regenerative Potential of in vitro-expanded chondrocytes by selectively removing senescent cells*

109. **Connor Wiegand**, Joseph Candiello, Prashant N. Kumta, Jay Hoying and Ipsita Banerjee. *Islet-mimetic Organoid Vascularization using Microvessel Fragments*

110. **Jacqueline Wittmer**, Andrew Hudson, Andrew Lee, TJ Hinton, Daniel Shiwarski, Josh Tashman, Sai Gopal Yerneni, Phil Campbell and Adam Feinberg. *Perfused 3D Printed Collagen Tubes Support Tissue Viability*

111. **Piyumi Wijesekara**, Daniele Evangelista-Leite, Konrad T. Rajab, Philipp T. Moser, Kentaro Kitano, Konstantinos P. Economopoulos, Daniel E. Gorman, Harald C. Ott and Xi Ren. *Metabolic Labeling and Chemoselective Functionalization of Native Biomaterials*

112. **Saigopalakrishna S**. Yerneni, Ezgi Yalcintas, Emrullah Korkmaz, Jason D. Smith, O. Burak Ozdoganlar and Phil G. Campbell. *Transdermal Delivery of Extracellular Vesicles Using Dissolvable Microneedle Arrays to Control Inflammation*

113. **Michael Sippel**, Jaclyn Yracheta, Bijaya Parida, Margaux M Salas, Ben Antebi, Vijay S Gorantla, Eric K Weitzel, J Chen, T Swenson, Alejandro Almarza and Juan Taboas. *Novel Delivery System of TGF B-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa)*

Presenter is in **bold**

Monday, March 11, 2019 5:00-6:00 pm (odd numbers), 6:00-7:00 pm (even numbers)
Tuesday, March 12, 2019 5:00-6:00 pm (all numbers)

A Therapeutic Role for Profilin 1 in the Progression and Metastasis of Renal Cell Carcinoma

Abigail Allen (1), Ryan Martin (2), Walter Storkus (1,3,4,5,6,7), Michael Lotze (1,3,7,8,9) and Partha Roy (1,5,10)

(1) Department of Bioengineering, (2) Department of Biological Sciences, (3) Department of Immunology, (4) Department of Dermatology, (5) Department of Pathology, (6) University of Pittsburgh Cancer Institute, (7) Graduate Program in Microbiology and Immunology, (8) Department of Surgery, (9) Department of Health Sciences, (10) Department of Cell Biology, University of Pittsburgh

Malignant tumors of the kidney (Renal cell carcinoma: RCC) is a lethal form of cancer that accounts in 2018 for 63,000 new cases and 15,000 deaths in the US. Abnormal neovascularization by angiogenesis is a hallmark change of tumor microenvironment in clear cell RCC (ccRCC, where genetic loss-of-function (LOF) of Von Hippel Lindau factor (VHL) leads to abonormal upregulation of VEGF, a prominent pro-angiogenic growth factor). ccRCC, the most common subtype of RCC, (found in >75% of patients) has a 10% five-year survival rate in advanced disease1. Though current anti-angiogenic treatments involving VEGF signaling blockade are initially effective, they are not effective long-term due to development of intrinsic resistance. Therefore, an improved mechanistic understanding of dysregulation of tumor microenvironment could pave the way for next-generation therapies for ccRCC patients. Studies from our and other laboratories have demonstrated that Pfn1 plays an indispensable role in angiogenesis-promoting activities (migration, proliferation, pro- angiogenic factor secretion) of vascular endothelial cells. Pfn1 expression is upregulated in RCC, most prominently in the vascular tumor microenvironment, and high Pfn1 expression correlates with an advanced stage of cancer (high grade, metastatic propensity) and poor overall survival (OS) and disease-free survival (DFS) of RCC (including ccRCC) patients. These findings suggest that there may be a causal relationship between Pfn1 upregulation in EC, altered tumor microenvironment and ccRCC progression, and therefore, chemical inhibition of Pfn1 may be an effective strategy to curb RCC progression. I hypothesize that Pfn1 promotes ccRCC progression through stimulating tumor angiogenesis and limiting immune responses, which can be targeted by novel small molecule approaches. We will investigate the effect of pfn1 inhibition on proliferation and migration of RCC by in vitro assay using ethidium homodimer stain and time-lapse imaging, respectively. Additionally, RCC migration will be investigated by T cell transwell invasion of endothelial cell/RCC cell monolayer.

Synergistic interactions between PD1 and LAG3 limit anti-tumor immunity

Lawrence P. Andrews (1), Sasikanth Manne (2), E. John Wherry (2), Creg J. Workman (1) and Dario A.A. Vignali (1,3)

(1) Department of Immunology, University of Pittsburgh, (2) Department of Microbiology and Institute for Immunology, University of Pennsylvania, (3) Tumor Microenvironment Center, UPMC Hillman Cancer Center

Targeting PD1 with monoclonal antibodies has yielded clinical success for a variety of tumor types, yet overcoming further inhibitory receptor (IR)-mediated tolerance is essential to improve cancer immunotherapeutic responses. LAG3 co-expresses with PD1 on CD8+ tumor-infiltrating T cells (TIL), signifying a highly exhausted phenotype and blockade of LAG3 is being explored as a novel combinatorial immunotherapy with PD1 to reverse T cell dysfunction. Dual PD1/LAG3 blockade in C57BL/6 mice reduces B16F10 melanoma tumor growth compared to single monotherapies. As CD8+ TIL is the highest LAG3-expressing TIL population, it is hypothesized that PD1 and LAG3 synergize to limit CD8+ TIL function controlling antitumor immunity.

To understand the cellular and mechanistic basis for PD1/LAG3 synergy, conditional knockin mice “surgically dissect” Pdcd1 and/or Lag3 floxed alleles restricted to CD8+ T cells expressing E8ICre.GFP. B16F10 was utilized to understand how PD1 and LAG3 on CD8+ T cells mechanistically control tumor growth.

Pdcd1L/L E8ICre.GFP and Lag3L/L-YFP E8ICre.GFP mice show attenuation of B16F10 tumor growth, which is further enhanced in Pdcd1L/L Lag3L/L-YFP E8ICre.GFP mice improving survival. Loss of PD1 increases CD8+ TIL frequency, which is further enhanced with additional loss of LAG3. These T cells are not antigen-specific as assessed in a B16-gp100 tumor model but CD8+ TIL increase following PD1/LAG3 deletion is due to enhanced turnover as CD8 proliferation (Ki67/BrdU) increases yet survival (Bcl2) is reduced within the TIL. T cell polyfunctionality is increased in Pdcd1L/L/Lag3L/L-YFP E8ICre.GFP mice, with enhanced IFNγ and GzmB release. Other IRs (TIM3, TIGIT, 2B4) that normally co-express with PD1 maintain levels to compensate for PD1/LAG3 loss.

Overall, PD1 and LAG3 on CD8+ TIL limit antitumor immune effects as deletion of both IRs results in reduced B16-F10 tumor growth with enhanced CD8+ TIL functionality, suggesting that the development of LAG3 targeting agents in the clinic would yield improved responses with anti-PD1

Utilization of Superparamagnetic Nanoparticles and Columns for One-Step Purification of Recombinant Adeno-Associated Viral (AAV) Vectors

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Isolating pure viral particles is a crucial step in the field of gene therapy, but commonly used laboratory or kit-based protocols are lacking in efficacy, timing, or accessibility. Magnet-Activated Cell Sorting (MACS) has been shown to be an excellent method of selectively isolating cells; but an analogous protocol for vectors has not been attempted thus far. Superparamagnetic nanoparticles and magnetic columns can be used for quick and efficient one-step purification of recombinant Adeno-Associated Viral (AAV) vectors providing a versatile alternative to iodixanol or kit-based purification.

We used Miltenyi nanobeads and columns to determine if we could selectively bind and elute AAV2 out of cell lysate solution. We used a primary-secondary antibody combination to bind viruses to nanobeads. We ran the nanobeads through MACS columns, washed with PBS, and then rinsed with elution buffer. We collected the nanobeads by removing the columns from the magnets and washing with PBS. We used qPCR and SDS-PAGE to determine the presence of virus.

Results: The qPCR results of the antibody-coated well experiments show that AAV2 binds to the antibody and can be eluted off using 1M NaCl or 4M $MgCl_2$.

Based on both qPCR and SDS-PAGE, it appears a significant amount of virus flowed through during the initial flow through and wash steps, suggesting suboptimal binding.

The discrepancy between the two experiments may arise from the use of a secondary antibody in the column; they may not bind as efficiently as a single antibody.

We laid out the groundwork for a vector-MACS purification protocol, but revisions are needed. Specifically, our MACS binding step needs to be optimized by using a better antibody. A single step isolation protocol will ensure rapid acquisition of purer product.

Biomagnetic Genetically Programmed Multicellular Microrobots

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Untethered, controllable microrobots for performing minimally invasive medical interventions within difficult to access fluidic environments are of interest in regenerative medicine and biomedical engineering. Here, we demonstrate a synthetic biology-based system for controlling self-assembly of hybrid cellular-magnetic microrobots. To form the microrobots, Escherichia coli cells are transformed with an inducible genetic construct for expressing mCherry on the outer membrane of the cells, fused to the C-terminal end of an lpp-ompA surface display protein scaffold. The mCherry displayed on the cell surface acts as a crosslinker between the cells and anti-mCherry, antibody-conjugated paramagnetic microparticles. The surface display construct is placed under control of the PBAD promoter, allowing induction of the surface-displayed protein by arabinose. To form a hybrid biomagnetic microrobot, antibody-functionalized microparticles are incubated in a shaker for one hour with an arabinose-induced, overnight culture of E. coli. A polymeric material is formed upon crosslinking between mCherry on the cell surface and anti-mCherry antibodies on the microparticle surface. The resultant biomagnetic polymer can be magnetically manipulated within polydimethylsiloxane (PDMS) fluidic environments to propel and steer the microrobot. This external magnetic control enables the transport of macroscale cargo (e.g., acrylonitrile butadiene styrene spheroids) to a target site within the fluidic environment by the microrobot. Using this method, programmed cells responding to chemical cues in their environment can form aggregates and facilitate manipulation of objects at least nine orders of magnitude in volume greater than an individual cell. We expect this system to have applications delivering new constituents to the microbiome.

Administration of alpha-Klotho systemically enhances skeletal muscle regeneration

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One consequence typical of the aging process is an impaired ability of myofibers to regenerate and restore their original architecture following an acute injury. This reduced regenerative capacity can result in loss of muscle mass and mobility, leading to declining function and increased morbidity for an elderly population. Coinciding with this decline in regenerative capacity, studies have shown a gradual systemic decline in the anti-gerontic protein alpha-Klotho. This age-related decrease in alpha-Klotho has been shown to have deleterious effects on cognition, muscle strength, and endurance. Our findings have also demonstrated an important role for alpha-Klotho in the skeletal muscle regenerative cascade. Following acute injury, we have found that young mice exhibit a marked upregulation of Klotho within skeletal muscle. However, this upregulation diminishes with age. In this study, we evaluate the restoration of myofiber regenerative capacity in old mice through the administration of alpha-Klotho via two methods: intraperitoneal injection (IP) and adeno-associated virus injection (AAV). IP administration of the recombinant alpha-Klotho protein increased both local and systemic Klotho, and showed beneficial effects on myofiber regeneration and muscle function. However, the timing of administration relative to time of acute injury plays an important role in determining the response. Similarly, mice injected with AAV-Klotho showed upregulation of Klotho as well as beneficial effects on regeneration. These results suggest that alpha-Klotho delivery represents a novel therapeutic target to enhance skeletal muscle healing in an elderly population.

Sevoflurane is Associated with Worse Clinical Outcomes and Altered Inflammatory Markers in Blunt Trauma Patients: Potential Role of Single Nucleotide Polymorphisms rs4715332 and rs1695

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PURPOSE: Anesthetics can impact inflammation and have a role in the pathophysiology of traumatic injury. We hypothesized anesthetics may alter clinical outcomes following blunt trauma. The response to anesthetic sevoflurane can be impacted by two single nucleotide polymorphisms (SNPs), rs4715332 and rs1695. Both SNPs in linkage equilibrium: rs1695 on chromosome 6 and rs4715332 on chromosome 11. In a large, retrospective cohort of trauma patients, we examined potential associations among rs4715332, rs1695, systemic inflammation, and post-injury clinical outcomes.

METHODS: DNA blood samples from 453 blunt trauma patients were examined for 551, 839 SNPs using the Illumina® Infinium CoreExome-24 v1.1 BeadChip. Patients who received sevoflurane (+Sevo) were compared to patients who received an alternative anesthetic (+Other). Matched groups (based on age, gender, Injury Severity Score, comorbidities, heart rate and blood pressure) were derived using SPSS software and compared based on SNP and genotype. Control group was SNP rs7070005. 31 inflammatory biomarkers were measured using Human Cytokine/Chemokine MILLIPLEX™ Panel kit. Clinical outcomes assessed were length of stay in the ICU (ICU LOS), total hospital LOS (tLOS), requirement for mechanical ventilation, length of time on mechanical ventilation, and the Marshall Multiple Organ Dysfunction score (MOD Score). 31 cytokines and Marshall MOD Score were analyzed with two-way ANOVA and clinical outcomes with Mann–Whitney U test. Significance assessed at $P<0.05$.

RESULTS: MOD Score, ICU LOS, and tLOS were worse in +Sevo vs. +Other. Inflammatory mediators were altered significantly in +Sevo vs. +Other, with differences observed as a function of rs4715332 and rs1695 genotype. Control group rs707005 had no significant clinical outcomes though did have significance in inflammatory markers.

CONCLUSION: This is the first precision genomics study in the setting of traumatic injury and critical illness, and suggests that defined anesthetics impact clinical outcomes and inflammation both broadly and as a function of genotype.

In vitro optimization of hemoglobin solution for use in sickle cell hemoglobin replacement therapy

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Introduction: Sickle cell disease (SCD) is an autosomal recessive genetic condition in which mutant hemoglobin (Hb) pathogenically polymerizes within red blood cells (RBCs) especially under deoxygenated conditions. Intracellular Hb polymerization reduces RBC deformability, which in turn results in vaso-occlusion, hemolysis, hypoxia, and severe pain. As part of an ongoing effort from this lab to pioneer a novel cellular therapy in which pathological sickle Hb is replaced with healthy donor Hb within sickle RBCs, this work seeks to maximize Hb capture efficiency of donor Hb within RBCs using variations of concentrated donor Hb solutions and techniques.

Materials and Methods: Washed bovine or healthy human RBCs were lysed in sterile water and the free Hb purified and concentrated to obtain Hb solution at approximately 33-35 g/dL. Final Hb concentration and blood gas parameters were determined using hemoximetry (OSM3, Radiometer Inc.) and the ABL825 blood gas analyzer (Radiometer Inc.). Rheological properties (viscosity and elasticity) of Hb solutions were measured using the Vilastic-3 viscoelasticity analyzer (Vilastic Scientific Inc.). Hb solutions were serially diluted and used in our lab's proprietary intracellular hemoglobin replacement procedure for RBCs. Hb capture efficiency within RBCs was determined through mean corpuscular hemoglobin concentration (MCHC) calculation, where MCHC = total Hb / hematocrit.

Results: Thus far, this work has found a peak Hb encapsulation level of refilled RBCs to be 9.2 g/dL, however preliminary results indicate that increased donor Hb concentration improves Hb capture within RBCs. Using Hb solutions up to 40 g/dL and new RBC refilling techniques, we believe that Hb encapsulation closer to physiological levels are possible. The ability to adequately refill sickle RBCs with donor Hb will increase oxygen carrying capacity and prevent RBC sickling, which may be used as a potential treatment for SCD or alternative to chronic blood transfusion.

Understanding the role of the pleiotropic scaffolding protein IQGAP1 in Hepatocellular Carcinogenesis

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Hepatocellular Carcinoma (HCC) is the 5th most common cause of cancer-related death with an estimated 27,000 annual deaths in the United States. Current measures combating the disease are insufficient. A complete ectopic liver transplant is the only tool that reliably extends a patient's quality of life and survival, however the lack of donor organs limits this approach. Better understanding the underlying mechanisms that drive HCC oncogenesis is critical to develop effective therapies. Recently, we and others identified the scaffold protein IQGAP1 as a direct target gene of miR-122. IQGAP1 expression is elevated in 60-85% of HCCs, and it regulates numerous pathways associated with cellular proliferation, including Wnt and EGFR pathways. Therefore, our central hypothesis is that upregulated IQGAP1 promotes HCC tumorigenesis by activating pro-growth signals and inducing cellular proliferation.

To investigate how IQGAP1 affects proliferation we either knocked down or overexpressed IQGAP1 in HCC cell lines (Snu449, Hep3B & Huh7). We confirmed IQGAP1's role in the Wnt/b-catenin pathway using the TOPFlash reporter assay and expression of downstream Wnt target genes by qRT-PCR. IQGAP1 knockdown resulted in reduced expression of b-catenin targets, including CyclinD1 and Glutamine Synthetase. In contrast, IQGAP1 overexpression led to enhanced b-catenin activity and target gene expression. We next directly tested the role of IQGAP1 in proliferation using the EdU incorporation assay. IQGAP1 overexpression increased the percentage of EdU+ cells, and knockdown had the opposite effect, suggesting that IQGAP1 facilitates proliferation. Finally, to model IQGAP1 overexpression and to dissect its role in vivo, we utilized the Hydrodynamic Tail Vein Injection/Sleeping Beauty method to induce HCC tumor formation. Tumor formation was compared in mice injected with clinically relevant oncogenic drivers S45Y-b-catenin + MET (BM) with and without IQGAP1 (I) overexpression. Livers from mice injected with IBM were nearly twice as large as mice that received BM alone. Moreover, preliminary analysis suggests that, compared to BM mice, IBM-injected mice better modeled human HCC, as seen by increased expression of ?-fetoprotein and reduced miR-122. Taken together, our data suggest that IQGAP1 regulates proliferation, and one mechanism is via the Wnt pathway, making it an attractive therapeutic target for HCC. Our future studies will focus on identifying the key mechanisms by which IQGAP1 regulates hepatocellular carcinogenesis to develop novel, targeted therapies for HCC.

Molecular basis of Adipose-Derived Stem Cell (ASC) therapy for management of Radiation Induced Fibrosis (RIF)

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Radiation therapy is one of the most important tools in cancer treatment, however iatrogenic comorbidities including RIF can significantly impair patient healing and life quality. Several published case studies suggest that application of autologous adipose tissue aspirates and adipose stem cells at the irradiation sites can ameliorate RIF, though the mechanism is not clear. In this study, we evaluated the efficacy of adipose tissue aspirates and/or ASCs in management of radiation fibrosis in a mouse model and investigated the underlying molecular mechanism involved.

In vitro studies: Transwell co-cultures were performed using irradiated human foreskin fibroblasts (HFFs) and ASCs to determine fibrosis related genes that are down or upregulated in irradiated HFFs. Quantitative real time PCR was employed to determine gene expression. In vivo studies: Female C57BL/6 mice were irradiated with 35 Gray (Gy) at the flank region and monitored for expression of fibrosis related genes by quantitative RT PCR at days 1 and 14 post irradiations. Fibrosis was confirmed by histological analyses and range of limb motion measure. To assess ASCs intervention efficacy, irradiated mice were injected with adipose tissue aspirates from luciferase+ GFP+ mice at day 28 post irradiations. Potential of ASCs to mitigate the acute effects of radiation damage were investigated employing total body irradiation mouse model.

Successful development of RIF in C57BL/6 mouse model was confirmed by histological staining of collagens using Masson's Trichrome stain which confirmed collagens deposition at the site of irradiation. At day 14 post irradiations we observed an upregulation of fibrosis related genes TGF β (500 fold), CTGF (60 fold), Collagen1 (400 fold), Collagen3 (500 fold) and collagen4 (500 fold) as compared to non-irradiated mouse skin. We observed a loss of limb flexibility in irradiated mice and these mice showed range of limb extension to only 11.4 ± 2.7 degree as compared to 57.0 ± 2.5 ($p < 0.0001$) degree in non-irradiated mice. A single ASCs injection significantly restored the limb flexibility to 42.5 ± 2.5 degree ($p = 0.0013$). In addition, a single intraperitoneal injection of ASCs lead to a significant enhanced survival of 9.25 Gy total body irradiated mice ($P = 0.047$). Transwell cultures with ASCs demonstrated significant down regulation of pro-fibrotic genes in irradiated fibroblasts. We determined hepatocyte growth factor (HGF) as the key mediator secreted by ASCs in irradiated HFF co-culture. Further, addition of recombinant HGF significantly down regulated TGF β in irradiated HFFs. The empirical findings in this study provide a new mechanistic role of HGF secreted by ASCs in mitigating RIF and supports clinical observations that grafted adipose tissue has benefit for treating RIF. Further work will help to improve our understanding regarding the use of adipose tissue based cell therapy approach in treatment of already established fibrosis.

Making Cold Malignant Pleural Effusions Hot: Development of a Murine Model of MPE

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Background: Malignant pleural effusion from primary or secondary malignancy heralds advanced disease and poor prognosis. Most causes are secondary to primary malignancy in lung or breast cancer. Existing therapeutic options include thoracentesis, indwelling pleural catheter, chemical pleurodesis, surgical extirpation, chemotherapy, or radiation. Despite advances in palliative management, effective locoregional therapies have been outpaced by systemic treatments. The success of immunotherapy in lung cancer suggests that treatments may serve as novel therapeutic approach to treating patients with MPE. Oncolytic virotherapy, where genetically engineered viruses preferentially target and kill malignant cells, may eliminate disseminated cancer cells in metastases and MPE. We look to establish a murine model for MPE (M3PE), with hopes to assess the therapeutic impact of intrapleural vaccinia virus expressing cell surface bound IL-2 (vv-IL-2-CM) in MPE murine models (M3PE).

Methods: M3PE was created with injection of Lewis Lung carcinoma-red fluc (LLC) into the intrapleural space using anatomical landmarks. Tumor growth and metastatic spread was assessed using in vivo bioluminescent imaging. Varying concentrations of LLC was administered to establish an early versus late disease model. Mice were euthanized when moribund or at day 21, to assess evidence of gross disease upon mice necropsy.

Results: 80% of the mice had evidence of malignant pleural effusion in the late stage model (Day 14 post inoculation). Bioluminescent imaging confirmed evidence of unilateral pleural carcinomatosis disease in all models that received intrapleural injection. Upon murine necropsy for M3PE, there was evidence of contralateral pleural effusion, mediastinal disease and epicardial involvement. Mean M3PE survival was 21 days.

Conclusion: Previous studies have described malignant pleural effusion murine model. We seek to use our model with hopes to assess therapeutic efficacy of administration of combined IL-2 and checkpoint inhibition, and finally, vv-IL-2-CM in the presence of checkpoint inhibition and immunodepletion of NK cells after intrapleural administration.

MRTF/Profilin is an important signaling axis for metastatic outgrowth of triple negative breast cancer cells

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Treatment of triple negative breast cancer (TNBC) is particularly difficult due to lack of molecular targets. The vast majority of breast cancer patient mortality is a result of emergence of disseminated cancer cells from a so-called dormancy-like state allowing subsequent aggressive growth at the metastatic sites. Actin assembly and important regulators of actin assembly play a key role in promoting the dormancy to proliferation switch of extravasated breast cancer cells. MRTF (myocardin-related transcription factor – an important family of transcription cofactors for SRF)/SRF transcriptional axis plays a major role in transcriptional regulation of many major molecular components of actin cytoskeletal system. We hypothesize that MRTF plays a critical role in post-extravasation survival and outgrowth of TNBC cells. To test this hypothesis, we first performed matrigel-on-top assay (3D culture experiments which successfully predicts the post-extravasation pulmonary metastatic outgrowth competency of breast cancer cells) and found that loss-of-function (LOF) of MRTF, either by knockdown or small molecule inhibitor CCG-1423 (and its analog CCG-203971), significantly retards the outgrowth of isolated TNBC cells. In this model, LOF of MRTF also dramatically suppresses the progression of established outgrowth (an in vitro mimic of micro-to-macrometastasis progression) of TNBC cells. Translating these findings in vivo, in intracardiac-injection model of experimental metastasis, administration of MRTF inhibitor reduces the metastatic burden of TNBC cells in immunocompetent mice. These experimental data are consistent with clinical findings demonstrating significantly shorter progression-free survival with MRTF upregulation in breast cancer patients. To obtain further insight into the mechanism behind MRTF's regulation of metastatic outgrowth, we performed RNAseq from TNBC grown in 3D culture treated with CCG and identified a number of differentially expressed genes related to cell proliferation/survival and invasion. In addition to these findings, we also found that LOF of MRTF causes a dramatic reduction of intracellular content of actin-binding protein Profilin-1 (Pfn1), an important regulator of actin cytoskeletal dynamics. Using a tetracycline-inducible Pfn1 knockdown system, we further demonstrated that acute Pfn1 depletion alone is sufficient to cause a major reduction of metastatic colonization of TNBC cells in vivo. Collectively, these findings suggest that MRTF-Pfn1 is an important signaling axis for metastatic outgrowth of TNBC cells.

First-generation small molecule antagonists of profilin1 suppresses pathological retinal neovascularization

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Angiogenesis, a process of new blood vessel generation, is a cause of visual impairment and blindness in ischemic retinopathies. A drawback of the standard of care anti-angiogenic therapy targeting VEGF signaling is the development of therapeutic resistance in some patients. Another issue is that VEGF is also a survival factor for neuronal and normal vascular endothelial cells (EC). Therefore, there is a crucial need for identification of additional therapeutic avenues. We sought to identify small molecule antagonists of profilin1 (Pfn1), an actin-binding protein with well-established pro-angiogenic function, as novel classes of anti-angiogenic molecules with potential applications in ocular pathology. Non-cytotoxic first generation inhibitors of Pfn1:actin interaction were identified by computationally guided virtual screen of small molecule database followed by pyrene-actin polymerization biochemical screen and confirmatory proximity-ligation assays in vascular EC. Cellular F-actin level was assessed by rhodamine-phalloidin staining of cells followed by fluorescence quantification. Time-lapse imaging was performed to measure the average speed of random migration of cells. Cell proliferation was assessed by counting cells on different days in 2D cell culture. Matrigel-induced cord formation and mouse aortic ring assays were performed for in vitro and ex vivo angiogenesis studies, respectively. For in vivo angiogenesis, C57BL/6 pups subjected to Oxygen-induced retinopathy (OIR): 7-days-old (P7) were exposed to 75% oxygen for 5 days. Mice were returned to room air at P12 and injected intravitreally with inhibitor at P12 and P14. At P17, vascular tufts and ischemic area were evaluated on BS-1 lectin immunostained flat-mounted retina. Antagonist of profilin1 (Pfn1) inhibited EC proliferation, migration and elicited potent anti-angiogenic activity in vitro and ex vivo. In OIR model, Pfn1 expression was found to be upregulated in mouse retinas. Finally, intravitreal administration of small molecule antagonist of pfn1:actin interaction caused a prominent reduction in ischemia-induced pathological retinal neovascularization in mouse pups. In summary, these proof-of-concept studies lay a conceptual foundation for Pfn1-targeting next-generation anti-angiogenic agents for future development of new lines of therapies for certain retinal diseases that involve aberrant neovascularization.

Sex Differences in Oxidative Stress Response in Bicuspid Aortic Valve Aortopathy

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OBJECTIVE - The prevalence of aortopathy among bicuspid aortic valve (BAV) males is over twice that of BAV females. Sex differences are also present in degenerative thoracic aortic aneurysm (TAA) with females presenting one decade later than males. Susceptibility to oxidative stress has been demonstrated in BAV aneurysm-derived aortic smooth muscle cells (SMCs), though upstream effector pathways have yet to be elucidated. We hypothesize that the female sex hormone estrogen exerts a protective effect in human thoracic aortic SMCs through modulation of oxidative stress response, a key factor in the pathogenesis of BAV aortopathy.

METHODS - Demographics, imaging results and operative data were acquired from our database of over 900 patients and aortic specimens were collected from informed consent patients who underwent ascending aortic replacement, heart transplant or other cardiac procedures. Gene expression of estrogen receptors (ER) α (Esr1) and β (Esr2) and superoxide dismutase (SOD) isoforms 1, 2 and 3 in aortic media tissue from BAV and tricuspid aortic valve (TAV) patients was quantified using RT-qPCR. The effect of 17b-estradiol (E2) treatment on SOD expression was assessed via Western blot in cultured medial SMCs from the ascending aorta.

RESULTS - Among surgical patients with degenerative aneurysms, females were significantly older than males (67.8 vs 62.9 years, p=0.002) whereas there was no significant age difference by sex in BAV-TAA patients (56.7 vs 55.8 years, p=0.195). Esr1 and Esr2 gene expression in the aortic media was similar between the sexes and did not correlate with age or aortic diameter. Sod1, Sod2 and Sod3 were expressed in the aortic media independent of sex. SMCs isolated from female BAV-TAA patients and from healthy aortic specimens demonstrated an E2 dose-dependent increase in SOD3 expression (p=0.013, p=0.014). However, E2-mediated upregulation of SOD3 expression was significantly lower in SMCs isolated from BAV patients when compared to SMCs isolated from TAV patients (p=0.029). E2 treatment had no effect on SOD1 or SOD2 expression in either BAV or TAV SMCs.

CONCLUSIONS - Esr1/Esr2 and Sod gene expression levels in the aortic media were found to be independent of sex. Interestingly, SOD protein expression was less inducible in BAV SMCs than in TAV SMCs. Thus, altered response to E2-mediated oxidative stress modulation may influence BAV-associated aortopathy. These findings suggest a role for estrogen in the oxidative stress response in aortic SMCs that might explain clinical sex differences in BAV aortopathy. A translational approach, combining both clinical and cellular biology techniques, is the optimal method to delineate novel hormone-mediated pathways and potentially direct personalized pharmacologic therapies to manage BAV aortopathy in both sexes.

Adipose Derived Stem Cells Enhance Cardiac Function Preservation of a Biodegradable Cardiac Patch and Increase Vascularization in Rats with Subacute Myocardial Infarction

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Background: Although bioengineered cardiac patches provide not only mechanical support for ventricles but also induction of cardiac regenerative cells in the failing heart, the overall regenerative efficacy has been limited. On the other hand, adipose derived stem cells (ADSCs) have been promising cells for cardiac regeneration with strong neovascularization. We hypothesized that combined therapy with cardiac patches and ADSCs would enhance cardiac regenerative efficacy, compared to single therapy with cardiac patches or ADSCs in ischemic heart disease models.

Methods: Cardiac patches were generated from polyester carbonate urethane urea and porcine decellularized cardiac extracellular matrix. ADSCs (1.0×107 cells) constitutively expressing GFP were established from F344 rats and transplanted as a cell sheet over the left ventricle (LV) surface of F344 rats 3 days after left anterior descending artery ligation with or without an overlying cardiac patch. Cardiac function was serially evaluated using echocardiography for 8 weeks, comparing groups with combined cells and patch (C), ADSCs alone (A), patch alone (P) or sham groups.

Results: At 8 weeks post-transplantation, the LV wall thickness was higher ($P < 0.01$) and the percentage fibrotic area was lower ($P < 0.01$) in groups C and P compared with the other groups. The number of infiltrated cells into the patch was higher in group C vs. P ($P < 0.01$). Furthermore, vasculature in the peri-infarct zone was greater in group C vs. other groups ($P < 0.01$), and hepatic growth factor expression was higher in group C than group A and sham ($P < 0.05$). Interestingly, much greater numbers of engrafted ADSCs survived in the combination therapy vs. ADSCs therapy alone ($P < 0.01$). On echocardiography, LV ejection fraction ($P < 0.01$) and LV chamber size ($P < 0.01$) were both improved with the combination therapy as compared to the other groups.

Conclusion: ADSCs enhanced the therapeutic efficacy of a biodegradable cardiac patch and were associated with greater neovascularization in the peri-infarct zone following subacute myocardial infarction.

Treatment of a Mouse Model of Cholestasis with a Thyromimetic Improves Biliary Injury But Exacerbates Hepatocyte Injury

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Chronic cholestasis results from bile secretory defects or impairment of bile flow, and there are few effective medical therapies available. Thyroid hormone T3 and synthetic thyroid hormone receptor agonists are known to induce hepatocyte proliferation during liver regeneration. However, whether these drugs have therapeutic benefits in cholestatic liver disease is unknown. In this study, we administered GC-1, a thyromimetic that acts through the TR[?] receptor in the liver, to Mdr2 knockout (KO) mice, a model of sclerosing cholangitis characterized by bile acid (BA) regurgitation, periductular inflammation, and fibrosis. We determined Mdr2 KO mice fed 5mg/kg GC-1 diet had decreased bilirubin, liver to body weight ratios, serum alkaline phosphatase, but increased serum alanine aminotransferase and aspartate aminotransferase compared to KO mice fed normal diet as early as 1 week on diet. Histologically, KO mice on GC-1 diet had decreased ductular response, less bridging fibrosis, and fewer SOX9 positive hepatocytes compared to KO on normal diet. Although total liver BA were higher in KO mice fed GC-1 diet for 2 weeks compared to normal diet, they normalized to KO levels at 4 weeks of diet. To elucidate the mechanism of increased BA accumulation and liver injury, we examined expression of BA transporters and detoxification enzymes. KO mice on GC-1 diet had decreased bilirubin transport and detoxification genes, such as Mrp3, Cyp2b10, and Oatp4, compared to KO mice on normal diet, with the net result being retention of BA in the hepatocyte. Affymetrix gene array data also indicates that KO mice on GC-1 have normalized bile acid synthesis compared to WT expression levels. Thus, GC-1 reduces cholangiocyte injury during cholestasis by inducing retention of BA in hepatocytes, causing injury to the hepatocytes; this occurs through as-yet unknown mechanisms that are upstream of BA transporters and biosynthesis enzymes and appears to be [?]-catenin independent.

Strawberry Polyphenols are Intestinal Permeation Enhancers for Oral Drug Delivery

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Oral delivery of biologic drugs, especially therapeutic proteins, is highly desired due to its ease of use and improved patient compliance when compared with injections. However, successful oral formulations must overcome the impermeable intestinal barrier to reach the bloodstream and take effect. Many studies have demonstrated chemical permeation enhancers to improve the absorption of biologics through the intestinal epithelium. However, effective enhancers tend to induce cytotoxicity or otherwise damage the intestines. Given the lingering need for efficacious but well-tolerated permeation enhancers, this work presents the results of screening an extensive, food-base library for safe, natural chemicals that improve intestinal absorption of biologics.

Of over 100 crude food extracts prepared and examined, the vast majority did not induce cytotoxicity in intestinal cells. Additionally, most did not significantly change the barrier properties of Caco-2 epithelial monolayers, though some did significantly increase permeability. Of these, strawberry was the most effective and was chosen for further separation and investigation. Isolation of the polyphenol contents from the strawberry extract yielded components that were highly potent permeabilizers. Mechanistic studies on Caco-2 revealed that the strawberry polyphenols cause rearrangement of the cytoskeletal protein actin, as well as the tight junction protein ZO-1, both integral components to the epithelial barrier. When orally administered to mice, the strawberry polyphenols boosted the intestines-to-bloodstream transport of the model biologics 4 kDa and 40 kDa dextran by over 100%. Further, insulin administered orally, alongside strawberry polyphenol permeation enhancers, achieved twice as much time-integrated bioactivity as the current gold standard of subcutaneously injected insulin. These conclusive in vivo results demonstrate the ability of strawberry polyphenols to effectively increase epithelial permeability, enabling the oral delivery of macromolecular drugs. Future investigations will include delivery of a wider variety of protein drugs and comprehensive safety studies in mice and higher animal models.

The Role of Diploid Hepatocytes in Promoting Regeneration Following Acetaminophen Toxicity

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Acetaminophen (APAP), a commonly used analgesic/antipyretic, can cause acute liver failure and death when taken in excess. After acute liver injury from APAP, the liver can regenerate, and we recently show that diploid hepatocytes proliferate more rapidly than polyploid cells. Polyploidy is defined as an increased number of chromosome sets and affects >90% of hepatocytes in mice and ~50% in humans. While the regenerative ability of the liver is well documented, the role of ploidy is undefined. Therefore, we hypothesize that diploid hepatocytes are the main driver of liver regeneration after APAP injury.

To test our hypothesis, we injected 300 mg/kg APAP into liver specific E2f7/E2f8 double knockout (LKO) mice, which are enriched in diploids, and measured liver injury at 6, 12 and 24-hours post-injection. We found that LKO mice had lower ALT and AST serum levels compared to controls, and H&E staining revealed that LKO livers exhibited less centrilobular necrosis compared to control mice at all time points. Additionally, we observed more proliferating, PCNA+ cells in the LKO livers at 12-hours post-injury, suggesting that the diploids were promoting liver regeneration.

Conclusion: LKO mice are more resistant to APAP-induced injury due to increased hepatocyte proliferation driven by diploid enrichment.

Local Delivery of Adipose-Derived Mesenchymal Stem Cells Promotes Immunomodulation and Allograft Survival in Vascularized Composite Allotransplantation (VCA)

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Purpose: Adipose-derived mesenchymal stem cells (ASC) are promising for cell-based immunotherapy due to their minimally invasive harvest, high yield, and immunomodulatory capacity. Here, we investigated the effects of local vs. systemic ASC delivery on VCA allograft survival and alloimmune regulation.

Methods: Lewis rats received hindlimb transplants from BN rats and were administered donor-derived ASC (1×106cells/rat) locally (subcutaneous injection in the allograft) or systemically (intravenous) at postoperative day (POD) 1, combined with a short-term immunosuppressive regimen. Mixed chimerism, immune cell and cytokine expression in the lymphoid organs of recipients were measured by flow cytometric analysis. A secondary donor skin allograft was performed in long-term survival recipients at POD 200. The immunosuppressive function of donor-derived ASC was tested by mixed lymphocyte reaction (MLR) assay.

Results: Recipients that received local-ASC treatment achieved long-term (>160 day) allograft survival, whereas the average allograft survival time in the systemic ASC group or contralateral (non-transplanted) hindlimb local ASC group was 44 days and 13 days, respectively. Local ASC treatment induced donor-specific tolerance as indicated by the acceptance ofthe secondary skin allografts of same donor origin. Donor cell chimerism was detected in both local- and systemic- ASC treated recipients after transplantation. CD4+CD25+Foxp3+, CD4+IL-10+ and CD4+IL-4+ cells in blood of the local-ASC group was increased and persisted through 8 weeks after withdrawal of FK506, while CD4+IL-17+ and CD4+IFN-r+ cells were reduced in both local- and systemic-ASC groups. The higher amount of CD4+IL-10+ and CD4+IL -4+ cells were evident only in draining lymph nodes (DLN) of the local-ASC group, while no difference was observed in non-DLN and spleens among the three groups. MLR showed that ASC inhibited T-cell proliferation independent of ASC-T cell contact.

Conclusions: Local delivery of ASC promotes long-term allograft survival, modulates alloimmune responses in VCA, and holds certain advantages over systemic administration.

Manipulating Gene Expression of Human Lamina Cribrosa Cells and Astrocytes

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Introduction: Primary open angle glaucoma is a neurodegenerative disease that affects over 60 million people worldwide. It is more common in certain populations with prevalence rates of 2.4%, 3.6%, and 5.4% in those of European Descent, Hispanic Ethnicity, and African Descent, respectively. Previously, we have found that the microstructure and mechanical strain in lamina cribrosa (LC) is significantly different and that gremlin gene expression may be differentially regulated in these three populations. In this study, we aim to manipulate the gene expression of gremlin by delivering siRNA and shRNA via adeno-associated virus (AAV).

Methods: Human LC cells and astrocytes were isolated and cultured using an approved IACUC protocol. Immunostaining of gremlin was performed to examine protein expression in human LC cells and astrocytes. To evaluate the effect of transforming growth factor beta 2 (TGFβ-2) on human astrocytes, TGFβ-2 was added to the culture for 72 hours. To test AAV transfection efficiency, human LC cells and astrocytes were transfected with four different serotypes of AAV-CMV-GFP virus and the transfection efficiency was quantified by GFP signal.

Results: Immunostaining results show that gremlin protein is present in both human LC cells and astrocytes. Addition of TGFβ-2 reduces gremlin protein expression in human astrocytes. We found that both human LC cells and astrocytes are transfectable using an AAV virus, confirmed by GFP immunofluorescence.

Conclusion: Prior work in the literature has shown that Gremlin is upregulated in the LC region of glaucomatous tissues. Our data suggest that TGFβ-2 activity and extracellular matrix remodeling may contribute to the altered regulation of gremlin gene expression in optic nerve head astrocytes from donors of African Descent. We successfully delivered AAV into human LC cells and astrocytes and plan to examine the effect of gremlin knockdown on TGFβ-2 activity and extracellular matrix remodeling in future work.

Engineering universal CAR and SynNotch receptors for programmable antigen targeting

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Chimeric antigen receptors (CARs) are artificial T cell receptors that re-target a patient's T cells to specifically bind and kill tumor cells. Adoptive cell therapy with CAR T cells targeting CD19 has revolutionized treatment of refractory B cell acute lymphoblastic, and there is great interest in generating CAR T cells treating other cancers by targeting additional tumor antigens. Another promising class of engineered receptors are synthetic Notch (synNotch) receptors that can sense an antigen of interest on a neighboring cell and turn on the expression of any transgene(s) of interest. To expand the targeting capabilities of these receptors, we have developed "universal" CAR and SynNotch receptors whose antigen-specificity can be re-directed by co-administered tumor-specific antibodies. Instead of directly targeting a tumor antigen, our universal receptors contain the SNAPtag self-labeling enzyme, which reacts with antibodies conjugated to benzylguanine (BG) to post-translationally assemble complete antigen receptors. We demonstrate that the activation of SNAP CAR and SNAP-SynNotch receptors can be successfully re-targeted by several clinically relevant antibodies including: Rituximab targeting CD20, FMC63 targeting CD19, Herceptin targeting HER2, and Cetuximab targeting EGFR. SNAP-SynNotch cells demonstrated potent transgene activation, and SNAP-CAR T cells were capable of IFNgamma production and tumor cell lysis. In addition to directing antigen specificity, the receptor response was titratable by BG-antibody dose. Finally, a continuous mathematical model with parameter scanning was constructed to describe and optimize the dose-response behavior of the SNAP receptors. SNAP synNotch and SNAP CAR T cells provide a powerful new strategy to retarget engineered cells to multiple antigens and provides researchers with the ability to rapidly screen CAR and synNotch antibody candidates. As synNotch receptors have been previously shown to be active on a variety of human cell types including fibroblasts and mesenchymal stem cells they are of additional clinical interest in tissue engineering applications.

Bi-directional and diametrical regulation of mesenchymal stem cells and epithelial-mesenchymal plasticity in prostate cancers

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Tumor progression is dependent on the interactions between the tumor cells and the surrounding cells in tumor microenvironments. Mesenchymal stem (stromal) cells (MSCs) exist in many tissues and are known to be continuously recruited to and become integral components of the tumor microenvironment. It has become apparent that tumor-associated MSCs (TA-MSCs) have an active role in tumor initiation and metastasis. Targeting TA-MSCs upstream or downstream modulators or use MSCs as vehicles for the delivery of tumoricidal agents represent promising therapeutic approaches in cancer treatment.

Prostate cancer (PCa) cells, along with other cancers, display phenotypic plasticity between epithelial and mesenchymal to escape from the primary tumor and seed into the ectopic sites. Metastatic PCa cells undergo a mesenchymal to epithelial reverting transition (MErT) upon arriving at the ectopic organ (e.g. liver, lung and brain); this confers resistance to cell death induced by cytokines or chemotherapy. A secondary epithelial to mesenchymal transition (EMT) is required for the tumor outgrowth and re-emergence. However, it is yet unknown why drives these phenotypic switches.

Herein, we show the bi-directional and diametrical interactions between MSCs and different phenotype of PCa cells. Transwell co-culture or exosomes derived from epithelial (E-cadherin+) PCa cells increase MSCs migration. In return, direct or transwell co-culture of MSCs promotes epithelial PCa losing E-cadherin on the cell surface and disruption of cell-cell contacts, which marks EMT. The conditioned medium from MSCs is sufficient to drive EMT. On the other hand, co-culture with MSCs facilitates mesenchymal PCa cells to undergo MErT, re-express E-cadherin, ZO-1 and Connexin 43 on the cell surface. Further, the conditioned medium from MSCs and E-cadherin- PCa cells co-culture, rather than MSCs alone or with E-cadherin+ PCa cells, has capability of driving MErT. In a summary, epithelial and mesenchymal phenotypic switching during PCa metastasis display distinct role on MSCs recruitment, and educate MSCs to diametrically interact with tumor cells via paracrine signaling to regulate phenotype switching and tumor progression.

Exposure of muscle stem cells to a stiff microenvironment drives an “aged” mitochondrial phenotype

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Aging is typically associated with increased tissue stiffness due to fibrosis and abnormal extracellular matrix (ECM) deposition. Although it is widely appreciated that extrinsic mechanical properties have the potential to impact stem cell fate, we know little about how tissue stiffening associated with increased age affects stem cell function. Our previous study demonstrated that aging causes alterations in ECM mechanical properties in skeletal muscle, and that these changes drive fibrogenic conversion of muscle stem cells (MuSC) (Stearns-Reider, et. al., Aging Cell, 2017).

Here, we tested the hypothesis that aberrant ECM stiffness impacts MuSC mitochondrial structure and bioenergetics. We focused on mitochondrial phenotype and function given its potential role in fibrosis. We also focused on the effect of substrate stiffness on regulation of the longevity gene, Klotho, given our recent findings that Klotho plays a critical role for MuSC bioenergetics. Klotho also has previously reported role in the inhibition of signaling pathways associated with fibrosis (Zhou et. al. 2013).

First, to quantify how aging affects muscle stiffness, we performed biaxial testing on young and aged muscle, followed by finite element analysis. Our analysis reveals the Young’s Modulus (E) of aged muscle to be approximately four-fold higher than young muscle.

Next, we engineered PDMS substrates that mimics the stiffness of young and aged muscle. We then seeded the constructs with young MuSCs. Consistent with the reduced mitochondrial network size, bioenergetics, and Klotho expression in aged MuSCs (Sahu et al., Nat. Commun., 2018), we find that young MuSCs cultured on the stiffer (aged) PDMS display decreased mitochondrial network size, bioenergetics, and Klotho expression.

Taken together, these findings suggest that age-related increases in muscle stiffness may drive an age-like phenotype of mitochondria and mitochondrial dysfunction. These declines may be attributed to declines in Klotho expression. Our studies underscore the importance of skeletal muscle ECM stiffness on MuSC function and provide potential mechanism of myogenic-to-fibrogenic conversion of MuSC due to increased age.

Cardioprotective Actions of Relaxin via Wnt Signaling

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Background: Aging is major risk factor for cardiac arrhythmia and heart failure and is associated with increased cardiac fibrosis and reduced sodium current and action potential conduction. Relaxin, a hormone of reproduction, has substantial cardioprotective effects including suppression of age-associated arrhythmia and fibrosis through upregulating the voltage gated sodium channel, Nav1.5, and inhibition of TGFβ signaling, respectively. However, the mechanisms by which relaxin increases Nav1.5 and inhibits fibrosis are incompletely understood. Wnt signaling has been associated with altered Nav1.5 expression and activation of fibroblasts, resulting in increased collagen secretion. Here, we test the hypothesis that relaxin exerts its anti-arrhythmic and anti-fibrotic actions through interaction with canonical Wnt signaling.

Methods: Young (9-month-old) and aged (24-month-old) F-344 rats were used for their age-associated arrhythmia and fibrosis susceptibility and were treated with vehicle (sodium acetate) or relaxin (400 μg/kg/day) for 14-days. Further, isolated myocytes and fibroblasts were treated with relaxin(25 nM), TGFβ(2 ng/mL), Wnt1(0.1 µg/mL) or Dikkopf-1(0.1 µg/mL) recombinant proteins, and/or L-NAME(100 µM) or L-NMMA(257 nM), for 48 hours followed by immunofluorescence to measure Nav1.5, collagen and Wnt peptides.

Results: Aging resulted in significantly reduced β-catenin expression, and mis-localization of connexin-43 to lateral membranes in left ventricular tissue sections. Relaxin treatment reversed these effects while also increasing Nav1.5 expression. Relaxin significantly decreased expression of the canonical Wnt inhibitor, Dikkopf-1, and increased nuclear β-catenin compared to control, indicating activation of canonical Wnt signaling. Relaxin and Wnt1 recombinant protein significantly increased Nav1.5 expression. Importantly, isolated myocytes treated with relaxin or Wnt1 in combination with Dikkopf-1, or the nitric oxide inhibitors L-NAME or L-NMMA, showed significant block of relaxin and Wnt1's effect on Nav1.5 expression. In addition to the effects of Wnt signaling in cardiomyocytes, we show that relaxin blocked the TGFβ-mediated increase in collagen secretion by cardiac fibroblasts, an effect abrogated by Dikkopf-1.

Conclusions: These data show that aging results in reduced expression or mis-localization of proteins vital for cell-cell coupling and propagation of the cardiac action potential and that relaxin can reverse these age-associated pathological processes. Further, these data suggest for the first time, that relaxin's cardioprotective effects in aging are mediated by Wnt-signaling in cardiomyocytes and fibroblasts.

Using CyTOF to elucidate the signals controlling progenitor cells during liver regeneration

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The liver is the only organ known to have the capacity to regenerate a majority of its mass. One of the main signals that control liver size is the transcriptional co-activator YAP. Activation of YAP has been shown to induce a stem-like progenitor state and increase liver size. However, other signals that control this regenerative capacity are largely unknown. We propose to use a YAP-expressing mouse model and mass cytometry to analyze the various signaling pathways activated in these progenitor cells that contribute to their regenerative capacity. Through these experiments, we hope to gain a better understanding of how cells in the liver revert to a stem-like state and contribute to its regenerative capacity. This knowledge could be harnessed to aid in liver regeneration after injury.

AAV delivery of α-Klotho: Gene therapy as a strategy to counteract sarcopenia

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Beyond the role of skeletal muscle as a mechanical motor, the endocrine function of muscle and its critical role in maintaining organismal metabolic health is increasingly being appreciated. With aging, however, there is a steady decline in both motor and endocrine function of skeletal muscle, resulting in organism-wide pathologies, including sarcopenia. In the elderly, this loss of skeletal muscle mass and accompanying muscle strength translates to increased morbidity. Recently, the anti-aging hormone, α-Klotho, has been associated with increased activity levels and has been shown to play a critical role in skeletal muscle regeneration after acute injury. In addition, we have shown circulating levels of α-Klotho are inversely proportional with age, and studies report mice genetically deficient for α-Klotho exhibit declines in forelimb muscle strength, similar to that observed in their aged counterparts. While the functional declines are apparent in aged mice, we show that the sarcopenic profile is truly evident in geriatric (>26 months) mice, corresponding to the timepoint when α-Klotho is significantly diminished. In this study, we investigated the ability of AAV-vector mediated delivery of α-Klotho to reverse the effect of sarcopenia in aged and geriatric mice. We found that AAV-mediated delivery of α-Klotho to aged mice resulted in a significantly improved functional capacity; however, the improvement was diminished in geriatric mice. These findings are consistent with previous reports of anabolic resistance with advanced age, and suggest that the therapeutic window for α-Klotho administration may be dependent on age of the host.

Nitrite Regulates Mitochondrial Dynamics to Inhibit Vascular Smooth Muscle Cell Proliferation

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Restenosis following balloon angioplasty in treatment of atherosclerotic plaques is a clinical complication occurring in 10% of patients who undergo the procedure. Restenosis is marked by neointimal hyperplasia due to smooth muscle cell migration into the intima and aberrant proliferation. Vascular smooth muscle proliferation leads to decreased vessel luminal diameter in response to local secretion of inflammatory cytokines near the site of disrupted vascular endothelium. Recent evidence has shown that nitrite (NO_2^-) inhibits smooth muscle cell proliferation and attenuates restenosis after vascular injury. However, the mechanism of nitrite-dependent inhibition of smooth muscle cell proliferation remains elusive. Nitrite is an established regulator of mitochondrial morphology and function, and mitochondrial dynamics have been previously shown to regulate cell cycle progression. Thus, we hypothesized that nitrite modulates mitochondrial dynamics to inhibit cell cycle progression resulting in attenuation of smooth muscle cell proliferation. Primary rat aortic smooth muscle cells (RASMC) were harvested from 6-8 weeks of age Sprague-Dawley rats. Nitrite inhibited PDGF-induced proliferation of RASMC in vitro in a concentration dependent manner as measured by 3H-thymidine incorporation. RASMC treated with nitrite exhibited markedly higher mitochondrial fusion compared to control as observed via transmission electron microscopy. Concurrent Western blot analysis showed that nitrite upregulated expression of mitochondrial fusion protein mitofusin-1 (Mfn1). Further, nitrite treatment was shown to upregulate the cyclin dependent kinase inhibitor p21, consistent with cell cycle arrest. Genetic silencing of Mfn1 in RASMC via siRNA attenuated nitrite-mediated inhibition of PDGF-induced RASMC proliferation as well as upregulation of p21 levels. Ongoing studies suggest that nitrite inhibits Mfn1 degradation by the ubiquitin/proteasome system. These data elucidate a novel mechanism by which nitrite regulates Mfn1 to attenuate smooth muscle proliferation. These data have important implications for the utilization of dietary and pharmacological nitrite to attenuate restenosis as well as identify Mfn1 as a target of nitrite.

Oncolytic virus immunotherapy-induced remodeling of antitumor immunity is improved through vector-encoded metabolic modulation

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Background: Immunotherapy has shown impressive clinical responses, but many patients do not respond to single modality immunotherapy due to a number of non-redundant resistance mechanisms. Our lab and others have proposed that tumor cells compromise T cell function by generating a metabolically inhospitable microenvironment, suggesting that immune or tumor metabolism can be differentially modified to improve T cell responses and thus immunotherapy.

Methods: We identified the adipokine leptin as a means to remodel the metabolic state of tumor infiltrating T cells. To assess the effects of leptin in the tumor microenvironment, we generated an aggressive PTEN/BRAF melanoma line overexpressing leptin, as well as an oncolytic strain of Vaccinia virus engineered to induce tumor-specific secretion of leptin.

Results: Treatment of T cells with leptin in vitro resulted in dramatic metabolic reprogramming. In vivo, intratumoral administration of leptin resulted in enhanced T cell metabolic and effector function. We then engineered melanoma cell lines to locally secrete leptin. While there was no proliferation difference between wild-type and leptin-expressing tumor cells in vitro, these cells are controlled in vivo in a CD8+ T cell specific manner. Leptin overexpressing tumors have increased T cell infiltration compared to control tumors, and these TIL are metabolically and functionally superior. In order to translate out findings to a therapeutic setting we utilized an oncolytic virus model. Oncolytic viruses are an attractive therapeutic modality promoting tumor specific killing as well as inducing an anti-tumor immune response. While wild-type oncolytic Vaccinia resulted in some tumor regression, leptin-engineered Vaccinia had superior therapeutic efficacy. TIL from these tumors have improved T cell infiltration and function. TCR sequencing revealed that the influx of new T cells by vaccinia is characterized by a polyclonal repertoire. On the other hand, T cells from tumors treated with leptin-expressing virus showed a reduced polyclonal phenotype indicative of specific clonal expansion. This clonal expansion is associated with a more memory like state, and indeed leptin-engineered virus induced a greater percentage of CD127hi memory precursors than the control oncolytic virus.

Conclusions: Taken together, these data suggest metabolic modulators like leptin can be therapeutically exploited to bolster intratumoral T cell function using the oncolytic virus platform. Our goal is to further design novel therapeutic strategies using oncolytic viruses.

Lack of Beta-catenin in Hepatocytes Impairs Proliferation and Promotes Liver Progenitor Cell-Mediated Repair in Response to Hepatic Injury

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Liver regeneration is normally mediated by hepatocyte proliferation. If hepatocyte proliferation is impaired, biliary epithelial cell (BEC)-derived liver progenitor cells (LPCs) are activated and mediate regeneration by differentiating into hepatocytes. The choline-deficient ethionine-supplemented (CDE) diet model of liver injury is known to induce proliferation of LPCs, but does not block hepatocyte proliferation. Beta-catenin signaling plays an important role in liver regeneration by promoting hepatocyte proliferation. Therefore, we hypothesized that Beta-catenin loss in hepatocytes would impair hepatocyte proliferation and lead to BEC-derived LPC-mediated hepatic repair in the CDE diet model. To this end, we performed genetic fate tracing in mice by utilizing adeno-associated virus serotype 8 carrying thyroid binding globulin-driven Cre (AAV8-TBG-Cre) to simultaneously delete Beta-catenin and permanently label hepatocytes with EYFP (KO2 mice). Importantly, in this model BECs contain Beta-catenin and do not express EYFP. After two weeks of CDE diet, KO2 mice displayed increased liver injury and a lack of hepatocyte proliferation compared to Beta-catenin WT littermates. Finally, in KO2 mice allowed two weeks recovery on normal diet after CDE diet we detected clusters of hepatocytes which expressed Beta-catenin and did not express EYFP, indicating that they originated from the BEC compartment. We did not observe expansion of EYFP-negative hepatocytes in control mice where hepatocytes retained Beta-catenin expression. Furthermore, we performed positive lineage tracing using a BEC/LPC marker-driven Cre recombinase to label BECs/LPCs with EYFP. In these mice we utilized GalXC-CTNNB1, a drug containing anti-Ctnnb1 small interfering RNA (siRNA) conjugated to hepatocyte-targeting ligand, to knockdown expression of Beta-catenin specifically in hepatocytes (KO3 mice). KO3 mice on CDE diet followed by recovery showed clusters of EYFP-positive hepatocytes, indicating BEC/LPC differentiation to hepatocytes. Thus, our results support the hypothesis that LPCs mediate liver regeneration when hepatocyte proliferation is impaired.

Role of SLC25A34, an uncharacterized mitochondrial protein, in fatty acid metabolism and mitochondrial respiration in primary hepatocytes

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Liver regeneration and diseases associated with altered mitochondrial metabolism, including NAFLD and hemochromatosis, are associated with alterations in ploidy. Moreover, many genes that regulate mitochondrial metabolism are reported to alter hepatic polyploidy. SLC25A34 encodes an uncharacterized inner mitochondrial membrane protein, with a novel role in liver polyploidy. Interestingly, SLC25A34 is upregulated in livers from patients with NAFLD. We now hypothesize that SLC25A34 regulates various aspects of liver metabolism.

In the current study, SLC25A34 was overexpressed or knocked down in primary hepatocytes harvested from adult C57BL6 mice and subsequent effects on different metabolic phenotypes were studied. Downregulation of SLC25A34 significantly increased the lipid droplets with a concurrent increase in expression of key players of fatty acid metabolism (Cpt1A, Srebf1, Acly). Maximal mitochondrial respiration and spare respiratory capacity were significantly elevated, possibly attributing to the increased levels of lipid droplets. Also, basal mitochondrial respiration and ATP-linked mitochondrial respiration were significantly increased, accompanied by increased expression of regulators of mitochondrial biogenesis (PGC-1 alpha, PGC-1 beta, PRC and TFAM). In contrast, overexpression of SLC25A34 decreased expression of Cpt1A, Srebf1, Acly, but had no effect on lipid droplets. Unexpectedly, SLC25A34 overexpression also increased maximal mitochondrial respiration and spare respiratory capacity, as well as non-mitochondrial respiration. Brdu proliferation assay suggested SLC25A34 is necessary for promoting hepatocyte proliferation.

In summary, the data suggest that SLC25A34 is critical for maintaining homeostatic mitochondrial function/lipid metabolism and modulation of SLC25A34 creates distinct metabolic phenotypes in the primary hepatocytes. Future studies will be conducted to explore the mechanistic role of SLC25A34 in liver metabolism in vivo utilizing SLC25A34 KO mice.

Age-related declines in α-Klotho drive progenitor cell mitochondrial dysfunction and impaired muscle regeneration

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Aging is associated with an impaired capacity of skeletal muscle regeneration after an acute injury which results in declining functional mobility and is associated with an increased morbidity in the elderly population. While young muscle is capable of restoring the original architecture of damaged myofibers, aged muscle displays a markedly reduced regeneration. We show that expression of the “anti-aging” protein, α-Klotho, is up-regulated within young injured muscle as a result of transient Klotho promoter demethylation. However, epigenetic control of the Klotho promoter is lost with aging. Genetic inhibition of α-Klotho in vivo disrupted muscle progenitor cell (MPC) lineage progression and impaired myofiber regeneration, revealing a critical role for α-Klotho in the regenerative cascade. Genetic silencing of Klotho in young MPCs drove mitochondrial DNA (mtDNA) damage and decreased cellular bioenergetics. Conversely, supplementation with α-Klotho restored mtDNA integrity and bioenergetics of aged MPCs to youthful levels in vitro and enhanced functional regeneration of aged muscle in vivo in a temporally-dependent manner. These studies identify a role for α-Klotho in the regulation of MPC mitochondrial function and implicate declining α-Klotho as a driver of impaired muscle regeneration with age. Hence, α-Klotho can be a potential therapeutic target for enhancing skeletal muscle vitality of an aging population.

African spiny mouse (Acomys) regeneration following acute, chronic, and volumetric muscle loss injuries

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Regeneration is the perfect regrowth and repair of damaged tissue. It is nature's ultimate solution to wound healing. The African spiny mouse (Acomys) is the only mammal in the world capable of scar-free skin regeneration as an adult.

In order to study ear skin regeneration, we punched holes in the ears of Acomys as well as normal mice (Mus), which serve as nonregenerating controls. We observed that Mus simply scarred, whereas Acomys was able to regenerate hair, adipocytes, cartilage, and, most interestingly, skeletal muscle.

We sought to further characterize Acomys's ability to regenerate different types of skeletal muscle. The Tibialis Anterior (TA) leg muscles of the mice were injected with cardiotoxin, a snake venom derivative that damages muscle. It was found that regeneration occurs much faster in Acomys. Mus again showed substantial scarring, whereas no such fibrosis was present in Acomys.

Next, we sought to determine the extent to which Acomys is able to regenerate in response to repeated injury. After the initial injection, the mice were given 3 weeks to heal and then were injected again. This was repeated for a total of 5 injection-healing cycles. Amazingly, even after chronic insult Acomys was still able to regenerate its muscle perfectly.

We then looked to see whether Acomys could recover from volumetric muscle loss (VML) in which a portion of the muscle is removed. VML injuries are common in gunshot or car accident victims. To simulate VML, hole punches were made in the TA muscles of the mice. Preliminary data suggests that Acomys shows improved regeneration compared to Mus following VML injury.

The results of continued study of Acomys could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the healthcare field by allowing for the regeneration of muscle and other tissue types.

IL-6 and IL-8 drive IR-specific immune suppression of effector, memory and naïve, peripheral blood CD8+ T cells in cancer patients

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Background: Cancer patients that do not respond to PD1 blockade have increased inhibitory receptor (IR) expression in peripheral blood lymphocytes (PBL) and increased cytokine concentrations in the plasma. Cancer patients off therapy and with normal white blood cell counts are often at greater risk for infections, immune dysregulation, progressive disease or reactivation of viral infections. However, the exact mechanism of this systemic immunosuppression in cancer patients is not fully understood. We performed flow cytometric assays to assess both phenotype and function of peripheral CD8+ T cells in cancer patient samples and healthy donor controls. We hypothesize that cancer patients may have systemic immune suppression via cytokine-driven IR expression in all CD8+ T cells subsets, including naïve cells.

Materials and Methods: PBL were obtained from healthy donors and treatment-naïve NSCLC, HNSCC, and melanoma patients. IR (i.e. LAG3, PD1, CTLA4, etc) expression was assessed on CD8+ T cells, CD4+ T cells, and regulatory T cells. Cytokine concentrations were compared by Luminex between plasma from healthy donors and plasma from cancer patients with high and low IR expression on peripheral CD8+ T cells. Autologous micro-stimulation assays were performed on peripheral CD8+ or CD4+ T cells with antigen presenting cells plus or minus IR blockade.

Results: CD8+ T cells, including CD45RA+CCR7+CD62L+CD8+ T cells, from cancer patient PBL contain elevated total LAG3 expression which correlated with stage and elevated expression of other IRs. Further, CD8+ T cells from these patients had decreased proliferation, which was rescued with the addition of anti-LAG3 or anti-PD1. Plasma from these patients had significantly elevated levels of cytokines that can signal via STAT3 (i.e. IL-6, IL-8, IL-9), which were independently found to increase total IR expression in healthy donor, naïve CD8+ T cells.

Conclusion: The current understanding of PD1 blockade resistance has been limited to the tumor microenvironment (TME) and our findings support the growing body of literature that tumor-related systemic immune suppression is a potent mechanism of cancer progression. Patients with cancer have systemic elevations of cytokines that signal via STAT3 leading to increased IR expression in naïve, peripheral CD8+ T cells making them poised for exhaustion even before TCR binding. These findings suggest that IR blockade also plays a significant role in reversing immune tolerance outside of the TME and cytokine blockade may play a role in reversing PD1 blockade resistance.

Local Delivery of Adipose-Derived Stem Cells Promotes Allograft Survival and Durable Tolerance in Vascularized Composite Allotransplantation

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Background: Successful restoration of aesthetics and function have been realized in vascularized composite allotransplantation of the hand, face, penis, and upper and lower extremities, however long-term immunosuppression remains a major barrier for procedure implementation and poses considerable risks for non-life saving operations. Adipose stem cells (ASCs) have both regenerative and immunomodulatory properties and have previously shown promise for inducing chimerism and reducing immunosuppressive burdens in long-term rat models of VCA when systemically delivered. However, these immunomodulatory effects were only transient and resulted in long-term (>120 day) allograft survival for only 47% of the animals. We hypothesize that local delivery of ASCs will improve immunomodulation through paracrine and direct cell-cell contact. The purpose of this study was to compare graft rejection after VCA with systemic, local and contralateral ASC delivery and investigate potential immunosuppressive mechanisms of action.

Methods: In Vitro. Passage three donor-derived ASCs were cultured with combinations of IFNg and TNFa for 2h, 4h, 6h and 24h. Expression of immunomodulatory relative genes were examined with real-time PCR. In Vivo. Transplant donors were male Brown Norway (BN) and recipients were male Lewis (LEW), a full MHC mismatch. Anti-lymphocyte serum (day -4 and +1) and FK506 (day 0-21) were injected intraperitoneally into the recipient rats. ASC therapy included donor-derived P3 ASCs (1*106 cells/rat) locally or contralaterally (subcutaneous tissue of allografts) or systemically (intravenous) at postoperative day 1. Survival time was compared between treatment groups.

Results: In vitro, ASCs secrete high levels of anti-inflammatory factors such as PGE2, IDO and iNOS after 6-24 hours exposure to IFNg and/or TNFa. In vivo, local-ASC delivery significantly extended long-term (>130 days) allograft survival compared with systemically treated rats (median survival time was 44 days).

Conclusions: Local delivery of ASC significantly prolonged allograft survival time and reduced immunosuppressive burden. Secretion of IDO could play a vital role in the mechanism of action for ASC-mediated immunosuppression in VCA. Future studies will investigate conformational changes in cell surface proteins that induce innate immunity, inflammation and graft tissue damage in VCA grafts that are also ameliorated with ASC therapy.

The Angiogenic Capability of Mesenchymal Stem Cells Coupled with Tenascin-C Under Hypoxic Conditions

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Human mesenchymal stem cells/multipotent stromal cells (MSCs) have great promise in aiding wound healing through their secretion of regenerative paracrine factors, that recruit and coordinate additional cell types within the wound microenvironment. However, their clinical utility is significantly hindered by their poor survival rates post transplantation due to the harsh microenvironment in injured tissue. Previous work in our lab has shown that the matricellular protein Tenascin-C can provide signaling via the epidermal growth factor receptor (EGFR) by restricting its activation at the plasma membrane. This prolonged sequestration of EGFR results in enhanced pro-survival signals, via low level tonic Erk and Akt signaling. Our lab is now investigating how these proximal signals from TNC influence MSC-mediated paracrine signaling during the wound healing process. Here we further characterize the ECM-MSC signaling dynamic by comparing the relative expression of genes involved at different phases of wound healing under various oxygen growth conditions representing normoxia (21% O2), normal skin (4% O2), and skin wound micro-environment (1% O2). MSCs cultured on TNCs exhibited significantly higher expression of genes relating to ECM modulation and angiogenesis at normoxic and hypoxic conditions. Thus, we subsequently performed a series of functional angiogenic assays to further delineate MSCs role within the proliferative phase of wound healing. Preliminary data exhibited Tenascin-C-MSC conditions being able to better promote vessel formation over non-Tenascin-C MSC conditions. These results suggest coupling of Tenascin-C to MSCs as a promising tool for MSC therapy in the wound healing process.

Effects of Matrix-Bound Nanovesicles in Human Spermatogonial Stem Cell Culture

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Spermatogonial stem cells (SSCs) are at foundation of spermatogenesis and essential for male fertility. Cancer patients who undergo chemotherapy and radiation treatments encounter a significant risk of stem cell pool depletion which can lead to permanent infertility. Since prepubertal boys are not yet producing sperm, they can only preserve testicular biopsies. Prepubertal testicular tissues house SSCs which can be used in tissue-based or cell-based therapies to produce sperm in the future. SSC transplantation is a promising technology used to restore fertility. However, the number of human SSCs (hSSCs) recovered from a small testis biopsy from a young patient may be limited. Therefore, establishing a culture method to expand hSSCs in vitro is a crucial step toward cell-based therapy. Extracellular matrixes (ECMs) have been used as bioscaffolds in regenerative medicine to support survival and growth various cell types, in vivo and ex vivo. We recently reported that human testis ECM substrate in hSSC culture yielded a significantly higher number of undifferentiated spermatogonia during a 14-day culture period compared to STO feeder cells. The current study will specifically test the bioactivity of human testis ECM-bound nanovesicles (MBVs), which are known to carry microRNAs, cytokines, chemokines, and other proteins that that could impact survival, proliferation, and differentiation of hSSCs in culture. We tested MBVs derived from human testis, porcine testis, porcine urinary bladder and porcine small intestinal submucosa. We evaluated cultures using the high-throughput flow cytometry method that can simultaneously analyze multiple developmental stages of the cultured cells. Our preliminary data showed that MBVs are internalized by hSSCs. We did not observe differences among the MBV culture conditions in our initial 14 day culture experiments, but dosing studies are currently underway.

Mechanical Response of Chromatin to DNA Damage

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The DNA within the nucleus of a cell is packaged with histones and other proteins to form chromatin. Chromatin acts as a viscoelastic polymer network that displays power law behavior in response to perturbations including DNA damage and stresses applied through cell motility or mechanotransduction. The effects of DNA damage upon the mechanical properties of the chromatin network is a controversial topic in the literature of biophysics with some studies yielding results that support increased mobility of chromatin after damage is induced while other studies yield results that support decreased mobility. In this study, we implement a technique that allows for site-specific probing of chromatin in two distinct functional regimes. We utilize a particle tracking technique developed in our lab, know as Sensors of Intra-Nuclear Kinetics (SINK), to measure the Mean Squared Displacements of fluorescently labeled proteins bound to these distinct regions of chromatin and fit this to a power law model that allows us to extract biophysical parameters, such as force propagation and chromatin decondensation, that yield much more detailed information than simple comparisons of motion. We find differences in mobility between the two regimes in the absence of DNA damage. In the presence of DNA damage, the two regions display similar mobility. Lastly, we report more recent data comparing biophysical changes in the chromatin networks of three different breast cancer cell lines after treatment with two different chemotherapy drugs as well as differences between cell lines. Overall, this work begins to elucidate the mechanical properties related to the DNA damage response in different chromatin regimes, times post-damage, sources of damage, and cell lines.

Polyploidy in Liver Regeneration and Adaptation to Chronic Injury

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The liver contains diploid and polyploid hepatocytes (tetraploid, octaploid, etc.), with polyploids comprising ≥90% of the hepatocyte population in adult mice. The molecular mechanisms regulating polyploidization are well-characterized; however, it is unclear if diploid and polyploid hepatocytes function similarly in multiple contexts. Moreover, 5-60% of hepatocytes are reported to be aneuploid, as random chromosome gains/losses can occur during cell division by polyploid hepatocytes. To study the function of polyploidy and aneuploidy in the liver, we used mice lacking E2f7 and E2f8 in the liver (LKO), which have defective polyploidization. Diploid hepatocytes were enriched 20-fold in LKO livers, and nearly all LKO hepatocytes were euploid compared to control hepatocytes, which were mostly aneuploid, suggesting polyploid hepatocytes are required for producing aneuploid progeny. LKO livers functioned normally but became highly tumorigenic when challenged with tumor-promoting stimuli, suggesting that their tumors were driven, at least partly, by diploid hepatocytes capable of rapid proliferation. Indeed, LKO hepatocytes were more proliferative and out-competed control hepatocytes in competitive repopulation studies. To eliminate potentially confounding effects associated with E2f7/E2f8 deficiency, diploid and polyploid hepatocytes from wild-type mice were examined. Wild-type diploid hepatocytes also showed a proliferative advantage, entering and progressing through the cell cycle faster than polyploids. Finally, to investigate the role of diploids and polyploids in chronic injury LKO mice were bred onto a tyrosinemia background, a disease model where the liver can develop disease-resistant, regenerative nodules. Survival was significantly reduced in tyrosinemic LKO mice but they maintained the ability to form regenerative nodules. Molecular analyses revealed that the regenerative nodules were generated via aneuploidy and inactivating mutations. In summary we identified new roles for diploids and polyploids in liver function, demonstrating diploid hepatocytes are the most proliferative, and polyploid hepatocytes are required for the formation of aneuploid progeny and facilitate adaptation to chronic liver disease.

New theranostic approaches to chronic sterile inflammation and immune rejection monitoring and treatment

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Chronic inflammation and immune rejection are the most common causes of late-graft loss in solid organ transplantation and they are now an emerging threat for vascularized composite allotransplants (VCA) such as hand and face transplantations. Acute allograft rejection is mediated by T cells and this process is triggered by antigen presentation by antigen presenting cells (APCs) such as macrophages and Langerhans cells. Chronic rejection, on the other hand, is characterized by progressive interstitial fibrosis, intimal hyperplasia and narrowing of the vascular lumen (vasculopathy). Intimal thickening results from elaboration of the extracellular matrix, and recruitment of leukocytes in response to injury, acute rejection, or inflammation. Thus, preventing persistent or chronic inflammation may mitigate both acute and chronic rejection. In our previous studies, we demonstrated the anti-inflammatory efficacy of cyclooxygenase-2 (COX-2) inhibitor (celecoxib) loaded nanoemulsions (CXB-NEs) up to 72 hours in the Complete Freund's adjuvant (CFA) induced inflammation model (Clin.Imm 2015).

In this study, we show that NEs are uptaken by macrophages, which are pooled to site of inflammation, and can be detected up to 40 days post CFA injection. Macrophage infiltration at the inflammation site was monitored by NIRF whole body live imaging daily and then weekly after CFA injection. Animals were sacrificed after 40 days, both of the right and left paws were harvested, and tissues were prepared for histology with H&E staining, picrosirius red, immunohistochemistry, and immunofluorescence. Whole body live imaging and histological analyses showed that NIRF labeled NEs are internalized by macrophages, accumulated at the inflammation area, and maintain its anti-inflammatory effect up to the 40 days of single dose.

Based on our findings in the CFA model, we posit that this multifunctional theranostic platform can be useful in solid organ transplantation and VCA for reducing persistent inflammation and eventually preventing graft loss.

Foxm1 drives Cardiomyocyte Proliferation during Zebrafish Cardiac Regeneration

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Mammalian hearts cannot regenerate damaged tissue after myocardial infarction because adult cardiomyocytes (CMs) fail to sufficiently proliferate. During development mammalian CMs rapidly divide, becoming quiescent shortly after birth, and become post-mitotic as adults. Failure to replace damaged tissue with new CMs increases scarring and morbidity. Therefore, it is critical to discover mechanisms that regulate CM proliferation. Zebrafish offer insights into this because their CMs robustly proliferate after adult injury and have conserved homology with mammals; allowing for the identification of novel genes that increase CM proliferation. Previously, our lab performed RNA-seq on uninjured and amputated hearts at different stages to detect genes upregulated after injury. Using qPCR and RNAscope probes we identified during CM proliferation that foxm1, a forkhead-binding transcription factor, was upregulated 3 days post-amputation (dpa) when CM proliferation is first induced. Foxm1 is involved in cell division and is expressed in mammalian cardiac development, but its role in cardiac regeneration and subsequent CM proliferation have not been characterized. We hypothesize that Foxm1 is critical for CM proliferation, and that loss of its expression will promote increased scarring and delayed cardiac regeneration. To test this, we acquired the ENU-derived mutant zebrafish line foxm1-sa10708 (foxm1-/-) from ZFIN. We removed 20% of the ventricle via amputation, and extracted hearts at specific times to observe changes in CM proliferation (7dpa) and fibrosis (30dpa) using immunofluorescence (IF) and AFOG staining, respectively. We observed significantly decreased levels of CM proliferation in foxm1-/- hearts at 7dpa compared to wild-type (WT) hearts and we noted increased scar area in foxm1-/- hearts at 30dpa. We are also investigating which of Foxm1's downstream transcriptional targets are critical for regeneration and one target, cenpf, was also significantly upregulated after injury. We have acquired cenpf-sa12296 (cenpf-/-) mutants, performed the same surgeries on these fish, and found significantly increased scar area in 30dpa cenpf-/- hearts compared to WT hearts. We will determine if cenpf and foxm1 mutants share similar phenotypes across all stages of regeneration or if there are stage-specific roles for Foxm1's downstream targets. These preliminary findings indicate that Foxm1 and its transcriptional targets are necessary for CM proliferation after injury, and future experiments will be performed to understand the exact mechanisms they act through to promote proliferation.

Elucidation and Integration of Tissue-Specific, Protein-Level Inflammatory Networks following Vascularized Composite Allotransplantation

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Background: Insights into inflammatory events after vascularized composite allotransplantation (VCA) are critical to the success of immunomodulatory strategies of these complex procedures. We sought to use systems biology approaches to identify key dynamic networks and principal drivers of systemic inflammation following VCA.

Methods: Lewis rat recipients received either fully major histocompatibility complex-mismatched orthotopic hind limb VCA from Brown Norway donors along with therapeutic immunosuppression with tacrolimus (VCA+TAC) for 20 days, or control syngeneic transplants without TAC. Time-dependent changes in 27 inflammatory mediators were analyzed in skin, muscle and plasma using Principal Component Analysis (PCA), Dynamic Bayesian Network (DyBN) inference and Dynamic Network Analysis (DyNA) to define principal characteristics, central nodes, and putative feedback structures of systemic inflammation in each experimental group.

Results: DyBN of VCA+TAC rats data suggested a novel, central feed-forward loop involving VEGF and Leptin in muscle, with likely activation of the NLRP3 inflammasome (IL-1β, IL-18) and IL-6. In comparison, the response to syngeneic transplant was characterized by IL-18 downstream of VEGF. Systemically, DyBN suggested a feed-forward loop involving Leptin and the chemokines LIX and MCP-1 in VCA+TAC as compared to Leptin, LIX and RANTES in syngeneic control. The presence of IL-17A inferred from PCA in muscle and plasma of VCA+TAC but not in syngeneic control suggested a role for pathogenic Th17 cells. Similarly, DyNA revealed a complex inflammatory response that involves differential network connectivity and complexity in the VCA+TAC as compared to syngeneic control. We hypothesize that this spatiotemporal pattern represents repeated restimulation of inflammation that is countered by TAC locally, but which spills over into the systemic circulation.

Conclusion: Our studies define a complex spatiotemporal evolution of dynamic inflammation networks following VCA involving novel mediators such as Leptin. These approaches may help target Precision Medicine for an overall improvement in quality of life for VCA recipients.

Defining and Comparing Mechanisms of Uterovaginal Prolapse Repair Failure

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Pelvic organ prolapse (POP), a disorder signified by the descent of the pelvic organs into the vaginal canal, affects millions of women in the United States alone. The loss of structural support of the pelvic organs is cause by severe weakness or injury to the muscles and connective tissues of the pelvic floor. Of the 300,000 operations performed annually in the U.S., 70% will fail within seven years due to POP recurrence. Despite evidence of the use of synthetic mesh for POP repair being twice as effective in reducing POP recurrence, it has a 20% complication rate (e.g. pain, mesh erosion, mesh exposure).

Regenerative solutions show promise in enhancing the outcomes of POP repair. However, insufficient knowledge regarding the in-vivo conditions that lead to repair complications and failure has hindered their clinical application. Thus, in order to develop effective regenerative therapies that fulfill the functional demands of POP repair, these in-vivo conditions must be characterized. Therefore, the goal of this research is to quantitatively evaluate and compare mechanisms of POP repair failure following two common transvaginal surgeries for apical prolapse, native tissue repair and mesh-augmented repair, by measuring parameters of anterior vaginal wall descent—the most common failure site for apical suspension surgery for POP. These measures will provide estimates of the mechanical demand required of current POP repair procedures.

From the results obtained, the following research questions will be answered: 1) What are the anatomical contributors to failure after a native tissue and mesh augmented apical prolapse repair and 2) Which factors contributing to failure are most common among the two surgical procedures? These findings will aid the optimization of apical repair for uterovaginal POP by helping establish the functional design criteria and considerations of future regenerative solutions.

Pre-Dissection-Derived Geometric and Distensibility Indices Reveal Increased Peak Longitudinal Stress and Stiffness in Patients Sustaining Acute Type A Aortic Dissection: Implications for Predicting Dissection

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Background: Acute type A aortic dissection (TAAD) is a life-threatening condition with up to 50% mortality rate. Clinical guidelines recommend prophylactic surgical replacement of the ascending aorta at ≥5.5 cm to mitigate the risk of dissection, but up to 62% of patients with dissection have aortic diameters <5.5 cm. Biomechanical wall stress prediction models could help identify how these properties influence dissection and offer an opportunity for improved risk stratification and management of aortic disease.

Objective: Use pre-dissection non-invasive imaging to assess ascending aortic distensibility and build shape and distensibility-based patient-specific stress distribution maps in patients sustaining TAAD.

Methods: Review of charts from patients undergoing surgical repair of TAAD (n=351) led to a subset (n=7) with ≥2 pre-dissection computed tomography angiography scans and transthoracic echocardiograms (TTE) at least one year prior to dissection. Ascending aortic wall biomechanical properties (aortic strain, distensibility and stiffness) were measured by TTE and compared to non-aneurysmal and aortic-size matched controls with no history of dissection. Patient-specific aortic strain served as an input in aortic geometry-based simulated three-dimensional reconstructions to generate longitudinal and circumferential wall stress maps and inspection of day of dissection scans allowed for identification of primary tear locations.

Results: Pre-dissection echocardiography revealed ascending aortas of patients sustaining TAAD to exhibit increased stiffness when compared to both non-aneurysmal and aortic-sized matched non-dissected controls. Computation models generated stress contour maps revealing dissection patients to have increased longitudinal wall stress compared to non-dissected controls. There was no significant difference in circumferential wall stress. Pre-dissection ascending aortic stress models revealed overlap between regions of increased longitudinal wall stress and actual location of primary tear sites.

Conclusions: Using pre-dissection imaging, we identified increased stiffness and longitudinal wall stress in ascending aortas of patients whom ultimately sustained type A dissection. Patient-specific imaging-derived biomechanical property maps like these may be instrumental toward designing better prediction models of aortic dissection potential.

Modeling collagen fiber recruitment across the corneoscleral shell

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The collagen fibers of the eye have a natural waviness known as crimp. This crimp is the basis for the nonlinear biomechanical behavior observed at the macroscale, in a process of stretch-induced stiffening called fiber recruitment. Recently, we have shown that collagen crimp is not uniform around the globe. We hypothesized that such nonuniformity would affect the intraocular pressure (IOP)-induced collagen fiber recruitment across the corneoscleral shell, and in turn, the deformation of the globe. Our goal in this project was to test this hypothesis.

We constructed a 3D axisymmetric finite element model of the corneoscleral shell with a simplified optic nerve head. The corneoscleral shell was divided into seven regions: cornea and limbus, anterior-equator, equator, posterior-equator, posterior, peripapillary. Experimentally measured tortuosities for each region were used to determine the nonlinear hyperelastic properties, and the stretch-based collagen recruitment. We then simulated IOPs from 0 to 50 mmHg and quantified pressure-induced collagen fiber uncrimping and recruitment for the whole globe.

All regions exhibited sigmoid recruitment curves, but recruitment rates varied substantially. At low pressures, collagen fibers in the posterior equator were recruited the fastest, such that at a physiologic IOP of 15 mmHg over 90% of fibers had been recruited, compared with only a third in the cornea and peripapillary sclera. At an elevated 50 mmHg, collagen fibers in the limbus and anterior/posterior equator had been almost fully recruited (~ 100%), compared with ~ 90% in the cornea and posterior sclera, and ~ 75% in the peripapillary sclera and equator.

In summary, our simulation results suggest that collagen fibers across the corneoscleral shell are not recruited simultaneously, indicating region-dependent rate of tissue stiffening with IOP. Findings from this work may help understand the role of microstructure in eye physiology, aging, and in biomechanics-related ocular diseases, such as glaucoma.

Failure Biomechanics of Arterial Tissue

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Vascular tissue possesses remarkable biomechanical resilience enabling them to sustain billions of loading cycles over the human life span. Yet, rupture and dissection of the arterial wall are significant clinical problems with high rates of mortality and disability. Current surgical procedures in treating these diseases are adjudicated by weighing the increase in longevity against empirically based patient risk factors and surgical intervention risks. To improve upon these clinical guidelines, mechanistic knowledge of the vascular tissue mechanical failure is a must. However, the fundamental structure-biomechanical failure relationship for these tissues remain unknown.

We propose to quantify the structure-failure property relationship of the arterial wall tissue at two locations of the arterial tissue: cerebral artery and ascending thoracic aorta. Biomechanical rupture of the vessel wall is a significant clinical problem at both of these locations. First, we will investigate the rupture of intracranial aneurysms in cerebral arteries. The second disease of interest is acute dissection of the ascending thoracic aorta, the largest blood vessel. We hypothesize that the collagen and elastin fibers, the primary load-bearing components of the tissue, act as bridges at the tear tip to resist the tissue failure, and structural anomaly of these components lead to wall tissue failure. We will test this hypothesis by assessing the role of arterial tissue microstructure on its biomechanical failure properties utilizing an image-based experimentally validated multi-scale structural model for the aortic wall. This structural model was implemented using a custom nonlinear finite element code developed in our lab. We will use clinical imaging modalities to generate patient specific arterial geometries as well as multiphoton microscopy based imaging methods. We will use computational parametric studies to quantify how disease, through modification of the microstructure of the arterial wall, affects the arterial wall biomechanical integrity.

Characterization of Neurodevelopmental Defects Associated with a Mouse Model of Hypoplastic Left Heart Syndrome

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Introduction: Hypoplastic left heart syndrome (HLHS), a congenital heart defect involving left-sided heart structures, is associated with poor neurodevelopmental outcome, with >30% experiencing neurodevelopmental impairment. A mouse model of HLHS showed brain abnormalities, suggesting a shared genetic etiology for brain and cardiac defects in HLHS.

Hypothesis: Mutations in Sap130 and Pcdha9 causing HLHS in mice may individually or together cause brain abnormalities and neurobehavioral deficits.

Methods and Results: Confocal histopathology and MR imaging was used to analyze the brain in mutant mice that were double Sap130/Pcdha9 homozygous, single Pcdha9 or Sap130 homozygous, or with forebrain targeted Emx1-Cre deletion of a floxed Sap130 allele. Sap130/Pcdha9 and Sap130 mutants exhibited overlapping phenotypes with microcephaly, thin cortex, and defects in the olfactory bulb, cerebellum, corpus callosum, and hippocampus, structures commonly affected in HLHS. RNAseq of Sap130/Pcdha9 mutant brain showed dysregulation of genes mediating learning and memory, long-term potentiation, motor coordination, and feeding–processes often affected in HLHS. In the Emx1-Cre forebrain deleted Sap130 mice, forebrain hypoplasia was associated with cortical thinning and hippocampal dysplasia. DTI connectome analysis showed global reduction in neuronal connections. Homozygous Pcdha9 mutant mice, which are adult viable, showed only hippocampal dysplasia, but connectome analysis also showed global reduction in neuronal connections. In line with this, behavioral tests showed deficits in spatial memory acquisition and cued fear conditioning.

Conclusions: We showed HLHS associated Pchda9 and Sap130 mutations, individually or together, can cause brain defects with reduced neuronal connections and neurobehavioral deficits. These defects can occur independent of the cardiac lesion, indicating they are not of hemodynamic origin.

Quality by Design: methodology to boost process understanding in nanomedicine

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Although nanomedicine is a promising technology used to target immune cells and support regeneration, significant barriers to commercialization persist. We present a quality by design (QbD) approach successfully used to heighten process control before and during product formulation within the field of nanomedicine. QbD imparts quality by using risk management, design of experiments, and statistical modeling. We present two case studies that used a unique collection of regression methods to support the advantages of utilizing a QbD framework. In the first case, we aimed to formulate a highly concentrated celecoxib nanoemulsion. Failure mode, effects, and criticality analysis (FMECA) was used as a risk assessment to identify the process and composition parameters most likely to impact the nanoemulsion's critical quality attributes (CQAs), which included droplet size, drug loading, and shelf-life. The risk assessment identified the nature of factors and factor settings to be studied in a design of experiments (DoE). We applied a unique combination approach that compared multiple linear regression, boosted decision tree regression, and partial least squares regression to establish a process understanding control with respect to processing conditions and composition. Regression models revealed a stability tradeoff between proportions of a co-solubilizing agent and an imaging reagent. In the second case, we sought to identify a robust microemulsion platform and understand the processes that impact droplet diameter, polydispersity index (PDI), and colloidal stability. FMECA was used to make informed decisions as to the nature of factors and factor settings to be studied in a two-stage DoE. We applied a similar host of regression tools that identified a design space dependent only on composition and was robust to processing and scale-up conditions. In addition, the process controls implemented from the logistic regression enabled prediction of microemulsion colloidal stability from composition data. These two cases exemplify the benefits of quality by design.

Characterization of Multi-View Hemodynamic Data by Learning Mixtures of Multi-Output Regressors

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Multi-view data are an increasingly prevalent type of dataset that allows exploitation of relationships between distinct sets of variables. It is often important to analyze the correlation between pairs of views via multi-view component analysis techniques such as Canonical Correlation Analysis (CCA). However, different parts of the data may have their own patterns of correlation, the diversity of which CCA cannot reveal. To address this challenge, we propose a method called Canonical Least Squares (CLS) clustering. Somewhat like CCA, a single CLS model can be regarded as a multi-output regression model that finds latent variables to connect inputs and outputs. This method, however, also identifies partitions of data that enhance correlations in each partition, which may be useful when different correlation structures appear in different subsets of the data or when nonlinear correlations may be relevant. Furthermore, we introduce a supervised classification method that relies on CLS clustering. The value of these methods resides in their capability to find interpretable structure in the data to explain their predictions. Presently, central venous pressure (CVP) is considered an insensitive measure of a subject's intravascular volume status or its change. However, CVP is an essential component of the determinants of venous return to the heart. Thus, we reason that features of CVP should be informative in early identification of emerging changes of patient status e.g. due to bleeding. We demonstrate the potential utility of the proposed approach using an application in clinical informatics to detect and characterize slow bleeding in patients whose CVP is monitored at the bedside. We empirically show how the proposed method can help discover and analyze multiple-to-multiple correlations, which could be nonlinear or vary throughout the population, while retaining interpretability of the resulting models.

Leveraging Routine Blood Draws to Predict Risk of Hemorrhagic Shock Before Surgery

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Irreversible hemorrhagic shock (IHS), a condition associated with substantial blood loss and poor response to fluid resuscitation, can lead to organ failure, adverse cellular effects, and death [2]. Identifying patients prone to experience IHS could complement pre-operative assessment of surgical risk and guide resource allocation for closer monitoring and management.

We hypothesized that a subject's response to brief rapid blood loss would indicate their cardiovascular reserve. To test this hypothesis, we studied 36 healthy sedated Yorkshire pigs who first had a 20mL rapid blood draw during a stable period of 30 minutes, were then bled at 20 mL/min until mean arterial pressure (MAP) dropped to 30 mmHg, and fluid resuscitated. 10 pigs experienced IHS (defined as MAP<20 mmHg and requiring cardiopulmonary resuscitation despite fluid administration). Arterial, central venous and airway pressures were collected at 250Hz. We used a machine learning approach to identify subjects at high risk of IHS by analyzing sequential patterns that manifested in the hemodynamic waveform response during the blood draws. Sequential patterns were extracted using Graphs of Temporal Constraints [1] and a decision forest model was trained with these patterns to classify the subjects.

Evaluated in a leave-one-subject-out cross-validation, our method confidently identifies 30% of the subjects who experienced IHS (95% CI [15.6%, 44.4%]), while only giving on average one false alarm out of 10,000 such predictions. This model outperforms logistic regression and random forest models trained with statistically featurized data.

Using sequential patterns in hemodynamic waveform associated with pre-operative blood draws, we can predict which patients are prone to develop IHS in the course of surgery.

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This work is partially funded by NIH GM117622.

Kinetic Model Simulation and Antigenicity Analysis of a Miniaturized Fc-Binding Domain for Local Deposition of Antibodies

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Statement of purpose: Herein we described an injectable, affinity-based peptide coacervate with reduced antigenic potential called Z15_EAK. The peptide contains a miniaturized Fc binding domain, Z15 derived from Staphylococcus protein A (SpA), fusing with the self-assembling peptide EAK. The unique bi-functional peptide can be deployed for delivering therapeutic IgG antibodies in vivo. A unique bioinformatics approach was used to analyze antigenicity of the Fc-binding domain. A kinetic model was constructed to simulate the free fraction of antibody delivered in depots.

Method: Fibrilization and Fc affinity properties of Z15_EAK/EAK coacervate were examined with fluorescence-based assays, transmission electron microscopy, infrared spectroscopy, paper chromatography, and isothermal titration calorimetry. The effects of Z15_EAK on the kinetics of IgG retention in vivo was also monitored for 28 days in mouse footpads in which the materials were restricted in a confined space conducive for a more accurate fluorescent intensity measurement. A one-compartment kinetic model was built with Matlab Simbiology® module to predict the unbound IgG dissociated from Z15_EAK/EAK in the footpad depot. The potential of Z15_EAK to elicit inflammatory reactions was examined in vitro and in vivo. An Immune Epitope Database (IEDB) was used to predict class I and class II MHC ligands in Z15 restricted by 100+ frequently found HLA A, B, DR, DQ, and DP alleles, covering most of the population.

Result: Z15_EAK peptide was shown to possess fibrillizing property and Fc-binding function. The peptide induced a red shift in the Congo red absorbance characteristic of peptide fibrils, which were also observed in transmission electron microscopy. Titration of IgG against Z15_EAK indicates their interaction was specific and reversible, with calculated Kd in the low micromolar range. The coacervate was found to localize IgG in mouse footpads for up to 28 days. The in vivo data was fit to a one-compartment model for simulating the relative fractions of IgG dissociated in the depot. The model predicted close to 27% of the antibody injected were available as unbound for the duration of the experiment. Z15_EAK did not appear to induce acute inflammation or delayed reactions; injecting Z15_EAK into mouse footpads elicited neither interleukin-6 (IL-6) nor tumor necrosis factor-alpha (TNF-a) from splenocytes isolated from the animals one day, seven days, or eleven days afterward. The antigenic potential of Z15 was analyzed using a bioinformatic approach in predicting sequences in SpA and Z15 dually presented by class I and class II human MHC alleles covering the majority of the population. Potential T cell epitopes in SpA cross-reactive with known microbial antigens were removed by the miniaturization.

Conclusion: Z15_EAK is a potential platform for generating antibody depots by which the impacts of Fc-based biotherapeutics can be enhanced. The Matlab Simbiology® kinetic model simulation provides a rational basis for optimizing dosing regimens. The Fc-binding domain bioinformatics analysis may serve to reduce uncertainties in evaluating antigenicity of peptide biomaterials in general.

Defining transcriptional regulation of CD8+ T cells in HPV(+) and HPV(-) head and neck cancer via gene regulatory networks

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Introduction: Head and neck squamous cell cancer (HNSCC) accounts for 4% of all cancers in the United States, with increased survival in HPV(+) patients compared to HPV(-) patients. Previous single cell RNA sequencing (scRNAseq) studies from our lab demonstrated that HPV(+) and HPV(-) HNSCC have different transcriptional immune signatures, with significant differences being observed inCD8+ tumor infiltrating T cells (CD8+ TIL). However, what drives the differences in these transcriptomic profiles have not been well characterized. Identification of a transcriptional regulatory network would reveal unique mechanisms and pathways involved in HPV(+) and HPV(-) HNSCC CD8+ T cells.

Materials & Methods: scRNAseq was performed on viable CD45+ cells sorted from peripheral blood (PBL), tonsil, and tumor infiltrating lymphocytes (TIL). Alignments and library annotation was conducted by the analysis pipeline CellRanger on R. Quality controls, principal component analysis and clustering analysis were done by the Seurat R package on R Studio. A subset of scRNAseq data was separated for further gene regulatory network analysis. The computational method SCENIC (single cell regulatory network inference and clustering) was utilized to infer gene regulatory networks from co-expression analysis of scRNAseq and DNA motif analysis of HNSCC CD8+ T cells. The network activity of each regulon (a group of co-expressed transcription factors and genes) was calculated in each cell to identify the differentially regulated subnetwork of each cell population. The results from network activity calculation was utilized for further downstream analysis.

Results: Eight cell clusters were generated from HNSCC CD8+ T cells by tSNE-based clustering methods. The results generated cell clusters that reflected different network activities and clinical signatures associated with HPV status. HNSCC CD8+ T cells from TIL predominantly included two clusters, which were defined by several T cell exhaustion markers, including PRDM1 and BATF. In addition, pathway analysis was performed for each cluster by Reactome software, and interferon gamma signaling was differentially regulated in the same clusters predominant with exhaustion markers, whereas IL-4 and IL-13 signaling was differentially regulated in two separate clusters. These results will lead to further studies on gene and transcription factor regulators in HNSCC HPV(+) and HPV(-) CD8+ T cells.

Conclusions: Differentially regulated transcription factors and genes from specific subpopulations of HNSCC scRNAseq data can be identified by SCENIC. A integrated analysis combining differential expression from scRNAseq data, motif enrichment analysis, and gene regulatory networks could be used to trace transcription factor (TF)-specific gene regulations in HPV(+) and HPV(-) HNSCC. Futures studies on the mechanism of TF-specific gene regulations involved in these cell types could be leveraged to enhance immunotherapy in HNSCC patients.

Computational Assessment of Cardiac Hemodynamics and Biomechanics for a Torsional Ventricular Assist Device (tVAD)

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This study evaluates the effects of applied apical torsion on cardiac hemodynamics and biomechanics to determine design parameters for a torsional ventricular assist device (tVAD). Parametric computational simulations were performed on a patient-specific biventricular model attached to a closed-loop circulatory system using ContinuityPro (Insilicomed, Inc., La Jolla, CA). Varying levels of tVAD support were simulated by altering coverage areas and rotation angles. The resulting hemodynamics were compared to values of a clinical heart failure model. Principal stresses and strains were evaluated at the ventricular and tVAD base. Increasing coverage areas and applied torsion angles produced increases in ejection fraction, peak systolic pressure, and stroke work while simultaneously lowering end-systolic volume for both ventricles. Increasing these parameters also increased the resulting myocardial stresses and strains. These results indicate that the tVAD can potentially help restore cardiovascular hemodynamics toward healthier values. However, the resulting large deformations suggest the potential for myocardial damage.

Granularity and Parsimony of Hemodynamic Vital Signs Data Impact Accuracy and Timeliness of Assessment of Physiologic State

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Vital signs from 38 Yorkshire pigs were collected at 250Hz from: pulse oximeter, central venous catheter (CVC), arterial catheter (ART), and pulmonary artery catheter (PAC). The pigs were anesthetized and allowed to stabilize for 30min, then bled at a constant rate of 20mL/min.

Measured vital signs were used to compute derived features and train models to classify state (stable versus bleeding) using data at varying levels of granularity: low frequency (LF), heart-beat-to-beat (B2B), high-frequency (HF). Separate models were trained using different subsets of sensors (non-invasive-only, CVC, ART, PAC, ART+CVC, and ART+PAC). Models were trained either with knowledge of the pig's stable baseline reference (normalized) or without (non-normalized). Separate models were trained to optimize detection of positives (ongoing bleeding) versus negatives (stable non bleeding) at low (<10-3) false positive and false negative error rates, respectively.

Each model was trained using the random forest algorithm with hyperparameter optimization and cross-validated in a leave-one-pig-out framework. The stable vs. bleeding state classification performance is compared across models through a ROCOR curve, a variant of the Receiver Operating Characteristic (ROC) curve that combines log-scale ROCs for positive and negative detection performance. Detection lead time is compared through Activity Monitoring Operating Characteristic (AMOC) curves that present the tradeoff between False Positive Rate (FPR) and Time to Detection (TTD).

Our results show significant boosts in performance with the availability of a subject's stable state baseline. Non-normalized models perform poorly, suggesting the importance of personalized medicine in the fast and accurate detection of bleed. Further, increasing data granularity improves the performance for all models, in particular between B2B and HF models. Finally, our results show that data from the common CVC, when HF data and a baseline are available, ties as the top performing model in classification and timeliness of bleed detection.

Non-invasive Hemorrhage Detection Approach using Photoplethysmography

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Background: Many instances of significant bleeding may not occur in highly monitored environment, contribution in the delay in recognition and intervention. We proposed a non-invasive monitoring for early detection of bleeding using photoplethysmography (PPG).

Methods: Fifty-two Yorkshire pigs were anesthetized, stabilized and bled to hemorrhagic shock, and their invasive arterial blood pressure (ABP), and PPG data were collected [1]. Time series of vital signs were divided into data frames of 1 minute updated every 30 seconds and beat to beat features were computed. The final feature matrix contained 18 ABP features and 85 PPG features.
A supervised machine-learning framework using Least Absolute Shrinkage and Selection Operator regularized logistic regression model was constructed to score the probabilities for hemorrhage of each data frame. The data in stabilization period was set as normal control group and data in bleeding period was set as positive. Model performance was evaluated by receiver operating characteristic (ROC) area under the curve (AUC) with leave-one-out cross validation method, and its precision was assessed with activity monitoring operative characteristic (AMOC).

Results: Two different models were built using ABP and PPG features separately. The PPG model could classify the hemorrhage with AUC = 0.89, where the AUC of ABP model was 0.91. Also, the PPG model could detect the hemorrhage on average 15.5 minutes (equals to 300 ml blood loss) if the false alarm rate of 1% was tolerated, whereas the average detection time of ABP model were 12.5 minutes at same threshold of false alarm rate.

Conclusions: We proposed a novel non-invasive bleeding detection approach using PPG signals only. This method potentially can improve the identification of hemorrhage with in patients and environments where invasive monitoring is unavailable, such as outside the ICU or resource-limited settings.

Reference:
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Beating Heart Simulation of Left Ventricular Compression for Heart Failure

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Soft robotic sleeves for cardiac compression have recently shown promise. While these devices have been rigorously engineered with soft robotic principles, no investigations have looked at optimal force application to the heart and subsequent changes in hemodynamics and regional mechanics. We use multiscale cardiac simulations to analyze heart compressions and augmentation of cardiac function. These computational models combine high-order finite element analysis, myofiber architecture, a nonlinear constitutive law, and a dynamic model of myocardial excitation-contraction coupling. They are coupled to a lumped-parameter closed-loop circulatory model that allows simulations to achieve steady-state cardiac cycles. To simulate current axisymmetric compression sleeve designs, parametric studies are performed using a prolate spheroidal model that allows for many studies in which sleeve and myocardial parameters can be changed and solutions obtained rapidly. Initial compressions of a severe heart failure model (18% ejection fraction-EF) yield large increases in EF (31%) with modest increases in myocardial strains.

Analysis of the cross-linked ionic peptide, EAK16-II

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The ionic peptide EAK16-II has the potential to be used as an injectable biomaterial after optimization of the resulting fibril scaffold. Through the use of the chemical cross-linker N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) the glutamic acid (Glu, E) and lysine (Lys, K) residues can form an amide bond resulting from an SN2 reaction. The reaction can be examined using DRIFT IR and SEM. DRIFT IR spectroscopy showed an increase in amide I and II peak intensity from the cross-linked sample. The major increase in the amide II peak indicates an increase in peptide size, indicating fibril formation. SEM analysis was also consistent with fibril formation in the cross-linked samples over the pure EAK16-II. An average pore size of 0.609 μm was determined for the cross-linked sample while the uncross-linked sample has an average pore size of 1.01μm. With the addition of the EDC cross-linker, EAK16-II is able to form large fibril networks with decreased pore size. This cross-linked transport scaffold would provide new pathways to administering vaccines by carrying both adjuvants and antigens in the hydrophobic pores. The smaller pores would restrict diffusion out of the structure and result in a sustained release of antigen and adjuvant. This would allow for an extended activation of the immune system and produce additional antibodies over time, reducing the number of booster shots required for immunization.

A High-Density Electrode Array for Mapping Corticospinal Muscle Recruitment After Stroke

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Stroke is a leading cause of long-term disability in the United States. Although stroke mortality has decreased in recent decades, the rate of stroke-related disability is increasing. Common disability stems from muscle paresis or weakness that is due to corticospinal system damage. Motor retraining programs aim to restore lost function by strengthening corticospinal networks underlying movement; however, motor impairments are rarely ever fully resolved. Understanding mechanisms of motor impairment has the potential to inform rehabilitation practice. Previous work has shown altered corticospinal recruitment of impaired muscles, but the patterns of recruitment across a paretic limb are not well understood. We used a high-density electrode array (HDEA) to record electromyographic (EMG) signals from extrinsic hand muscles of the forearm in humans with long-standing paresis secondary to stroke. The HDEA, developed by our collaborators at Battelle, consists of 150 surface electrodes embedded in fabric that is wrapped around the forearm. The electrodes are stainless steel discs spaced approximately 15mm apart. Magnetic stimulation was applied to the scalp location overlying the optimal representation of the extensor digitorum communis muscle in primary motor cortex. Activation thresholds were established during various muscle states (rest and functional grasps) in stroke survivors and neurologically-intact controls. Stimulation intensity was graded according to threshold. Our results show that muscle state and stimulation intensity modulate the extent of recruitment. We also observed differences in activation patterns between stroke survivors and controls. The HDEA allows high-resolution mapping of recruitment across the limb and therefore may be used as a clinical tool to evaluate impairments and monitor training-induced changes during functional behaviors involving the upper limb. Our group is also exploring methods that deliver electrical stimulation through HDEA electrodes to provide stimulation-assisted movement. The knowledge gained from this project will be used to guide these methods and promote recovery from stroke-related disability.

Acute In Vivo Functional Assessment of a Biodegradable Stentless Elastomeric Tricuspid Valve

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Background: Traditional mechanical and bioprosthetic heart valve prosthesis have distinct disadvantages following implantation in patients. Biomimetic elastomeric Tissue Engineered Heart Valves (TEHVs) show promise in addressing these shortcomings by relying on biodegradable polymers to replace with native patient heart valve tissue over time.

Hypothesis: Evaluate the acute in vivo function, mechanics, and thromboresistance of a stentless biodegradable elastomeric TEHV in the atrioventricular position.

Methods: Biomimetic tricuspid valves were fabricated with poly(carbonate urethane)urea (PCUU) by double component deposition electrospinning. Valve geometry, leaflet thickness, and mechanical anisotropy were designed to recapitulate properties measured in porcine tricuspid valves. Five Yorkshire pigs underwent implantation of the TEHV in the tricuspid position with running 4-0 polypropylene suture and neo-chordal attachment. Post-implant epicardial echocardiography (echo) was performed prior to closure and extubation. All animals underwent final echo and valve explant at 24 hours. Explants underwent gross analysis, histology, and scanning electron microscopy (SEM). Valve leaflet thickness measurements and bi-axial mechanical testing were performed on explanted PCUU leaflets.

Results: Immediate post-bypass echo in the 5 successful implants demonstrated mobile valve leaflets without leaflet prolapse, with mild regurgitation in all cases. At 24-hours echo demonstrated good leaflet motion, no prolapse, and trace to mild regurgitation in all but one animal that showed moderate regurgitation due to an undersized valve. TEHVs demonstrated no thrombosis and retained structure. Histology revealed patches of proteinaceous deposits with no cellular uptake. SEM demonstrated retained scaffold fiber microarchitecture with no platelet aggregation. PCUU leaflet thickness and level of mechanical anisotropy were equal to pre-operative or native tricuspid measurements.

Conclusions: A bioinspired, elastomeric, stentless TEHV demonstrates good acute function and implantability in the tricuspid position. Scaffold-based TEHV technology shows promise as an improved solution for atrioventricular valve replacement.

Improving urinary catheter safety and tissue engineered urethral scaffolds though an enhanced understanding of human urethral biomechanics

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The male urethra is often subject to iatrogenic inflation injury during urinary catheterisation leading to acute treatment costs and chronic stricture disease. Treatment of the resulting stricture disease (and congenital/inflammatory urethral conditions) requires regenerative treatment approaches that tissue engineering can address using biodegradable scaffolds to restore damaged tissue. This study characterises the biomechanics of urethral tissue in order to improve urinary catheterisation safety and tissue engineered urethral scaffolds.

Nine human urethral samples were obtained from patients undergoing gender reassignment surgery. Samples were subjected to pressure-inflation testing to characterise tissue mechanics and an area of the urethra was subjected to urinary catheter inflation to examine injury thresholds. Sections of injured and non-injured urethra were stained for collagen, elastin and muscle. Planar sections of non-injured tissue were tension tested to characterise regional and directional variances in mechanical properties.

The urethral injury pressure values identified in this study have fed into the design of the TransUrethral Catheter Safety Syringe, a pressure relief safety device that attaches proximally to urinary catheters and decants inflation fluid if the urethral injury pressure threshold is exceeded thereby preventing iatrogenic inflation injuries during urinary catheterisation. This device has recently been trialled in 100 patients with the valve activating 7 times and requiring subsequent manipulation to allow for full inflation. The mechanical characterisation data generated has fed into the design of a more mimetic bi-layered tissue engineered scaffold comprised of bovine collagen and elastin configured in a dense desiccated film inner layer to prevent urine leakage and a porous freeze-dried outer layer to facilitate cellular infiltration. This material exhibits a closer mechanical and compositional match to native tissue than current gold standard tissue engineered constructs. Subcutaneous murine implants reveal increased cellularity and macrophage recruitment relative to gold standards thereby demonstrating the regenerative potential of this mimetic scaffold.

In-vitro flow characterization of PAAL & P-PAL using dimensional analysis for detection of abnormal flow conditions

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Acute and Chronic respiratory failure are leading causes of patient deaths in the United States. Current support systems prove to be cumbersome as they limit patient's ambulation and worsen long-term outcomes. The Paracorporeal Ambulatory Assist Lung (PAAL) is being designed to be a compact, wearable pump that can bridge lung failure patients to transplant or recovery, also providing ambulatory support. Acute in-vivo (6 hour) and 5-day evaluation of PAAL has been previously published and 30-day studies in ovine model are underway. Chronic in-vivo studies require diligent monitoring of the device parameters, mainly flow and torque, to continuously assess pump and bundle performance. Any abnormalities in flow might suggest possible thrombus formation, occlusion of cannula inlet/ outlet ports or kinking. An abnormal increase in torque can be an indicator of thrombus formation under the impeller. Though flow rates and torque are continuously monitored, their dependence on blood characteristics, primarily viscosity, make it difficult to distinguish any such abnormality from post-surgery animal recovery induced change in device performance. Hence, this study uses Buckingham Pi theorem to develop dimensionless flow and torque parameters as functions of angular velocity, whole blood viscosity, blood density and impeller diameter. A PAAL prototype was used to obtain pressure vs flow curves at various impeller rotation rates using Carboxymethyl-Cellulose (CMC) solutions of different viscosities. An empirical relation is obtained between the dimensionless parameters, which is then used to retrospectively evaluate the 30-day animal studies. Literature does not provide a reliable model for estimation of blood viscosity from Hematocrit (HCT). Viscosity measurements were performed using cone-plate rheometers on blood acquired from the in-vivo animals to obtain an accurate model for HCT-based viscosity estimation for dimensional analysis. Retrospective analysis of in-vivo data show that the dimensionless flow and torque parameters improve our ability to instantaneously determine thrombus/ occlusion effected changes in device performance.

Quality Assessment and Optimization of Nanoemulgels for Local Inflammation Treatment

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In response to injury, the body begins healing itself by first penetrating the site with M1 macrophages. M1 macrophages are pro-inflammatory; they work with the COX-2 pathway, which, when activated, leads to the production of prostaglandin E2 (PGE2). PGE2 activation creates a feedback loop that attracts more macrophages to the injury site. This loop leads to chronic inflammation when there is not a successful turnover from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages. This is a concern for regenerative medicine because it can affect how one's body responds to treatments and constant inflammation can also be a potential cause of rejection. Our lab has developed and demonstrated in animal models the use of theranostic nanoemulsions that deliver small molecule anti-inflammatory drugs to M1 macrophages to reduce PGE2 production and penetration of macrophages at the site of inflammation (Patel et al, Clin. Immunology 2015, Herneisey et al, Ther Deliv 2016, Janjic et al, JNI 2018). Theranostic nanoemulsions are both a therapeutic and a diagnostic tool, as we can use them to image drug delivery, release, and efficacy. The goal of this project is to make an injectable, thermo-responsive nanoemulgel for local control of inflammation in variety of injuries. We produced and tested pilot formulations of nanoemulgels, which were proven to be thermo-responsive, for their viscosity and colloidal stability. In our studies with rheology and dynamic light scattering, these thermo-responsive gels exhibit robust colloidal stability. Assessment strategies employed to optimize the formulation of nanoemulgels are presented along with computational methods for nanoemulgel further optimization.

Design and Manufacturing A Novel Customizable Nitinol-PTFE Stent Graft for Effective Torso Hemorrhage Control

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Hemorrhage control in the torso part is one of the substantial aspects in the patient survival. Many devices and treatment options have been developed to control internal bleeding, which help the surgeons in regulating the hemorrhage in the injured region with reasonable performance at the earliest possible time. We has been developed a novel customizable nitinol-PTFE stent graft for control the hemorrhage temporarily in traumatic hemorrhagic shock.

The graft consists of self-expanding nitinol stent, which is covered with highly-stretchable expandable polytetrafluoroethylene (ePTFE). We used micro-laser welding to connect nitinol wires in order to build up the metallic backbone. The welding process is followed by heat treatment to manifest the thermomechanical properties of the structure. The backbone is covered with thin-walled ePTFE tube that was stretched radially.

Several design parameters that govern the mechanical properties, biocompatibility, and functionality of the device for the hemorrhage control were investigated. We explored the effect of the welding parameters on the performance of the stent. Also, we studied different designs for the metallic backbone of the graft. The designs include different thickness of nitinol wires, backbone diameters, and distances between welding spots. The influence from heat treatment process was also studied for assessing the performance of the device, such as collapsing and deployment.

Various mechanical properties were characterized through in vitro tests to evaluate the device's performance. Various in vitro test results showed excellent hemorrhage control capability under the simulated pulsatile blood flow circulation. We optimized the design parameters to achieve the best performance of bleeding control. Our study results on a new stent graft for hemorrhage control confirmed that a highly-stretchable ePTFE covered nitinol stent could propose a new treatment option for rapid hemorrhage control for saving trauma patients.

Local Delivery of FK506 with Impregnated Nerve Wraps Accelerates Nerve Regeneration in Infraorbital Nerve Transection and Repair Model

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Purpose: Peripheral nerve injuries can be devastating, leading to permanent functional disabilities. Systemic FK506 administration has been shown to hasten recovery and improve functional outcomes after nerve injury repair. However, high systemic levels of FK506 can result in adverse side-effects. Localized administration of FK506 could provide the neuroregenerative benefits of FK506 while avoiding systemic, off-target side-effects. The purpose of this study is to infestigate the utility of a novel FK506-impregnated nerve wrap in treating peripheral nerve injuries in a previously validated rat infraorbital nerve transection and repair model.

Methods: Infraorbital nerve transection surgeries were performed on two groups (n=5 per group) of adult Lewis rats. In both groups, the infraorbital branch of the trigeminal nerve was transected. The transected nerve was then repaired primarily with 10-0 nylon suture with (treatment group) or without (no treatment group) the addition of a Poly(ester urethane) urea (PEUU) wrap impregnated with 20 mg of FK506. To evaluate neuroregeneration, trigeminal ganglion cell recordings, objective sensory testing, directional sensitivity, maximal response, and receptor compositions were analyzed from five rats in each group at four and six weeks postoperatively. Recordings from the trigeminal ganglion in naïve rats were taken for comparison. To assess local FK506 administration, blood and tissue samples (infraorbital nerve, muscle) were analyzed for FK506 concentration using liquid chromatography-mass spectrometry at four and six weeks postoperatively in the treatment group.

Results: Data were analyzed using custom software written in Excel Visual Basic and the Excel add-on statistical package, Analyze-it (Analyse-it Software, LTD). Peristimulus time histograms (PSTHs) having 1 ms bins were constructed from spike times of individual single units. Responses to stimulus onsets (ON responses) were calculated during a 20 ms period beginning 1 ms after deflection onset; this epoch captures the initial, transient phase of the whisker evoked response. Rats within the treatment group (FK506 wraps) were found to have increased response magnitude at 4 weeks after implantation in the infraorbital cut and repair model in comparison to no treatment group ($p<.013$, Fig. 1). FK506 blood levels at 4 and 6 weeks were close to the limit of quantification (<2ng/ml), whereas concentration within the tissues of interest, the infraorbital nerve and muscle, were much higher.

Conclusion: This study investigates the use of an FK506-impregnated PEUU nerve wrap to improve functional recovery following peripheral nerve injury. Sensory testing provides objective data on the effects of these wraps in the treatment of peripheral nerve injuries and the FK wraps appear to accelerate nerve recovery at 4 weeks, with minimal systemic drug exposure. The findings from this study may translate into novel treatment systems and protocols to treat nerve injuries.

A Novel Retrievable Rescue Stent as a Comprehensive Solution to Non-Compressible Traumatic Hemorrhage

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Introduction: Mortality after vascular injuries of the torso remains nearly 80% in both military and civilian settings. Even Resuscitative Balloon Occlusion of the Aorta (REBOA) has failed to significantly improve survival, perhaps due to ischemic complications. We hypothesized that a novel retrievable three-tier Rescue stent would provide thoracic and abdominal hemorrhage control while preserving critical visceral and spinal perfusion to improve outcome as compared to REBOA.

Methods: Retrievable stents were fashioned from laser welded nitinol wire and polytetrafluoroethylene. Ten swine were subjected to a thoracic aortic injury followed by either REBOA (n=6) or Rescue Stent (n=4) amidst hemodynamic and neurologic monitoring. Stents and balloons were recovered within 1 hour. Animals were resuscitated with the goal of survival and monitored until post-operative day 2.

Results: There were no differences pre-operatively between the REBOA and Rescue groups in baseline mean arterial pressure (MAP), hemoglobin, and lactate. The average intraoperative blood loss in the REBOA group was 3675 mL vs 1495 mL for the Rescue group ($P<0.05$). MAP was significantly lower in the REBOA group at 20 minutes (36.8 vs 63.8 mmHg, $P<0.05$) and at the end of the procedure (17.2 vs 46 mmHg, $P<0.05$). All REBOA animals showed profound spinal cord ischemia under neurologic monitoring and expired from profound malperfusion. Conversely, all Rescue animals survived the surgery neurologically intact and without evidence of organ malperfusion, with only one animal lost from an arrhythmia post-operatively.

Conclusions: Use of the Rescue stent is associated with significant reduction in blood loss, hypotension, organ malperfusion, spinal cord ischemia, and mortality as compared to current balloon occlusive approaches. This study suggests that the Rescue stent offers improved outcomes for non-compressible hemorrhage due to effective hemorrhage control with preserved visceral/distal perfusion. This rapidly delivered technology may offer new hope for these highly mortal injuries.

Development of a Novel Rabbit Surgical Model of Pelvic Reconstruction for the Use of Testing an IL-4 Eluting Coated Polypropylene Mesh

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Pelvic organ prolapse (POP), a disorder in which the muscles of the pelvic floor are weakened over time, affects over a million women each year in the United States. A quarter of these women undergo a reconstructive procedure, increasingly using polypropylene mesh as mechanical reinforcement to the pelvic floor. However, complications including chronic pain, mesh erosion, or exposure have been observed at rates as high as 10-20%. Previous studies have determined that the early macrophage polarization profile following biomaterial implantation is a strong indicator of overall tissue integration downstream. Recent work from our laboratory in developing a cytokine delivery system has shown that actively controlling the immune response to implanted mesh resulted in enhanced integration. Therefore, we present a rabbit surgical model to implant and investigate the host response to mesh into two different sites, including the vagina and the abdomen and scale up of a methodology to alter this response using a clinically relevant size mesh implant.

Commercially available polypropylene mesh was used to investigate the modulation of the immune response. An adapted radio frequency glow discharge method is used to create a stable negative charge on the surface of the mesh, followed by the sequential deposition of polycationic and polyanionic polymers. Interleukin-4 (IL-4), an immunomodulatory cytokine known to promote the M2 pro-remodeling macrophage phenotype, is incorporated into the coating to be released in a controlled manner upon implantation.

Utilizing a novel surgical technique in New Zealand white rabbits, we implant mesh analogously to human implantation and evaluate changes in the immunologic response at early (14 days) and tissue remodeling outcomes at late stages (90 days) of implantation. The mesh-tissue complex was removed from each rabbit and processed for histological analysis as well as immunolabeling of immune cells, such as macrophages.

We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive IL-4. We evaluated the biological functionality of the coated mesh via bioactivity studies and in vivo implantation. An ideal mesh would provide mechanical support to the pelvic floor while decreasing the inflammatory response and increasing integration with the surrounding native tissue.

An E-Mag Cannulation Approach Reduces Radiation Exposure During Simulated Fenestrated Endograft Repair

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Introduction: The evolution of fenestrated endografts continues to expand endovascular options into challenging branched regions of the aorta. Enthusiasm of these new endografts is tempered, however, by increased procedural time, and more importantly, significantly increased radiation exposure to staff and patients. The “E-mag” system was designed to expedite cannulation of endograft branches. Each fenestration is encircled by a copper coil that when energized attracts a magnetically tipped wire (Mag-wire).

Methods: The fenestrations of a custom nitinol PTFE fenestrated stent-graft were encircled with coils of copper wire, which were energized to create a local electromagnetic field. Current was applied to one fenestration at a time to magnetize each of the fenestrations. A Mag-wire was constructed from a neodymium magnet affixed to a standard 0.035 Glidewire. A mock aortoiliac system was constructed. Imaging was completed on a fixed fluoroscopic unit. Five surgical clinicians of varying experience were timed using either a standard Glidewire or the E-mag system for time to cannulate a branch fenestration.

Results: The magnetic field created at each fenestration averaged 1.5 Gauss. Submersion in an aqueous bath led to no appreciable temperature change or electric voltage leakage. Within the aortic model, the Mag-wire was attracted only to the fenestration being energized. The average time to cannulation with the Glidewire was 34.37 +/- 39.91 seconds while the average time using the E-mag system was 5.73 +/- 2.67 seconds ($p < 0.01$).

Conclusions: This feasibility study suggests that an E-mag system enhanced cannulation of fenestrations in a model of complex endovascular aneurysm repair. Continued development of this approach may allow reduction of radiation exposure during complex endovascular procedures.

Immobilization of Anti-Platelet Molecules on Implant Materials

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Over 600,000 Americans are killed by coronary heart disease annually. Balloon angioplasty is a commonly used minimally invasive procedure used to reopen the lumen by compressing encroaching plaque against the walls of the artery. During this procedure, a vascular stent is inserted to prevent the blood vessel from experiencing restenosis, but the force induced during the procedure has been shown to damage the endothelial lining of the vessel. To combat thrombotic clot formation patients are typically assigned an oral anti-platelet medication for up to 6 months following the procedure. However, older patients assigned an oral, and therefore systemic, anti-platelet medication become bleeding risks in the case of another surgery being performed on the same patient. This research attempts to address the problem of thrombotic clot formation by immobilizing an anti-platelet medication, Ticagrelor (TIC), on the surface of vascular stents and vascular stent material. Self-assembled monolayers (SAMs) are first formed on the oxide layer of low carbon stainless steel 316 (SS316L) stents, providing uniform coverage of the stent with amine functional groups presented at the surface. These functional groups are then exposed to TIC and crosslinking agents to covalently attach the medication to the surface. Surface immobilized TIC is expected to inhibit the surface activation of platelets contacting the damaged tissues around the stent, thereby inhibiting the formation of thrombotic clots.

Acute In Vivo Performance of a Pediatric Ambulatory Artificial Lung

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Respiratory failure is a significant source of pediatric morbidity and mortality. Current means of respiratory support typically render patients bedridden which can worsen long-term patient outcomes. Our Pittsburgh Pediatric Ambulatory Lung (P-PAL), and second generation Pediatric Modular Extracorporeal Lung Assist System (P-ModELAS), are integrated pediatric pump-oxygenators that enable ambulation. Our device is intended for long-term use and designed to provide up to 90% of respiratory support in children weighing 5-25 kg. This study aims to characterize the device performance in an acute ovine model.

The functional difference between the devices is a shortened blood flow channel connecting the pump and bundle in the P-ModELAS. Both prototypes use a centrifugal pump and a cylindrical, stacked fiber bundle (0.3 m2). In vivo device performance was evaluated in 6 acute (4.5 – 6 hours) studies using 23-32 kg sheep. A thoracotomy was performed to place a venous cannula in the right atrium and an arterial cannula in the pulmonary artery. The cannulas used varied in the first 4 studies as we refined our implant strategy. Oxygen transfer rates were measured at blood flows from 1 to 2.5 L/min. Bundle resistance, plasma free hemoglobin, and animal hemodynamics were measured throughout the experiment. ACT was maintained between 1.2-4.1 times baseline.

There was no statistical difference between the P-PAL and P-ModELAS performance. Oxygen transfer rates ranged from 39.9 – 83.4 mL/min (Hb = 6.2 ± 0.3 g/dL) at blood flows of 1 – 2.5 L/min. Blood flow in one study was limited due to a venous cannula occlusion. Changes in the implant strategy remedied this. The plasma free hemoglobin range was 5.8 – 10.4 mg/dL. Macroscopic evaluation of the bundle post-study showed small thrombi in four studies. One device failed due to poor priming. Based on our successful acute studies, we will move to chronic animal studies and further evaluate device hemocompatibility.

7-day In Vivo Performance of a Low-Flow Extracorporeal CO2 Removal Device

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Study: Extracorporeal CO2 removal (CO2R) devices have been found to significantly benefit patients with ARDS or acute exacerbations of COPD. These systems operate similarly to ECMO, but with the specific goal of removing CO2 from the blood at blood flow rates below 1 L/min. Our Modular Extracorporeal Lung Assist System (ModELAS) is an integrated pump-lung with 3 distinct respiratory support applications including adult low-flow CO2 removal. The CO2R configuration of the ModELAS is intended for 7-days of use and designed to remove 35 - 50 % of the metabolically produced CO2. This study aimed to characterize the in vivo performance of the device in a 7-day ovine model.

Methods: The ModELAS consists of a centrifugal pump and a cylindrical, stacked fiber bundle (0.65 m2). In vivo device performance was evaluated in 40 - 60 kg sheep (n=3). The 15.5 Fr Hemolung dual lumen catheter was placed in the right external jugular vein via a surgical cut down. The animals were recovered and tethered within a pen. The targeted blood flow rate was 0.5 L/min. Animal hemodynamics were measured hourly and CO2 removal was measured daily. Blood chemistry and plasma free hemoglobin (pfhb) were measured 5 times during each study. The target ACT was 1.5 – 2 times the baseline ACT and was achieved via continuous heparin infusion.

Results: All animals survived the study duration. Two animals had elevated creatinine and BUN levels on post-operative day (POD) 3. Both biomarkers were within the normal range on POD 6 suggesting an acute renal issue. One animal became anemic due to an axillary hematoma unrelated to the device or surgery. Pfhb ranged from 7 – 58 mg/dL. Elevated pfhb occurred in one animal with elevated kidney biomarkers before normalizing by POD 7. Average CO2 removal was 72 ± 5 mL CO2/min. One device contained thrombus in the bundle and at the pivot-bearings, however the ACTs during the study were below the target range 25% of the time. All other devices were free of thrombi.

Conclusions: These studies supported favorable results with positive low-flow CO2R performance of the ModELAS.

Polyelectrolyte multilayer coating for delivery of IL-4 from contact lenses for dry eye disease

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Purpose: Dry eye disease, while exceedingly common, has few treatment options outside of poorly absorbed eye drops that target only limited aspects of the immune system. Interleukin-4 (IL-4) has been shown to polarize macrophages from the inflammatory M1 phenotype - the phenotype prevalent in dry eye disease - to the anti-inflammatory M2 phenotype. IL -4 can be incorporated into a nanometer thick coating for mitigation of the foreign body reaction to implantable polypropylene mesh; however, application to other devices remains unclear. We hypothesize that this coating can be applied to silicone-hydrogel-based contact lenses with the goal of a sustained release profile of IL-4 for potential treatment of dry eye.

Methods: Senofilcon A lenses were rinsed to remove residual storage buffer and then dipped into polymer solutions of opposite charges (dermatan sulfate complexed to IL-4 served as anion and chitosan served as cation) in order to build up 40 bilayers of IL-4 containing coating. Immersion in alcian blue dye for 30 minutes followed by distilled water rinse was used to visualize surface coating. Coated lenses were incubated in solution containing chondroitinase ABC and chitosanase enzymes (or in solution void of enzymes) to mimic in-vivo conditions, and supernatant was collected and refreshed at various time points for 28 days in order to determine IL-4 release profile and kinetics using ELISA.

Results: A uniform and conformal blue stain remained on lenses dipped in the oppositely charged polymers (when compared to a control, non-dipped lens), showing successful application of polymeric coating to the lens. IL-4 release kinetics from a coated lens incubated with enzymes showed a sustained release of IL-4 over days vs no signal from an uncoated control lens. Interestingly, there was very little release of IL-4 from a coated lens in the absence of enzymes, showing that our coating is degraded primarily by enzymatic means.

Conclusions: Our results support the hypothesis that our polymeric IL-4 releasing coating can be applied to contact lenses with a resulting sustained release of drug over days vs the transient burst release seen with eye drops. Future in-vivo work is necessary to determine if these coated lenses can help to mitigate inflammation associated with dry eye.

Characterization of Floating Impeller Phenomena in an Integrated HFM bundle and Centrifugal Pump Design

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The Modular Extracorporeal Lung Assist Device (ModELAS) is a modular extracorporeal pump-lung capable of providing full respiratory support for adult and pediatric patients as well as low-flow CO2 removal for adults. Therefore, pump operation varies from .25-4 LPM flow. The ModELAS uses a magnetically coupled impeller with contact bearings that balance the magnetic coupling and hydrodynamic forces for stability. Proper balance of the downward magnetic and upward hydrodynamic forces are required to prevent excessive bearing wear and hemolysis. The goal of this study is to determine whether the forces balance the impeller in the ModELAS for all three respiratory applications while using a blood substitute with a viscosity of 3.5 cP. If forces are not balanced, an impeller with appropriate magnetic strength will replace the current impeller to prevent damage to the device or the blood. To characterize the force profile button compression load cells, placed behind the top and bottom ultra-high weight polyethylene bearings and zeroed before device priming, register a voltage signal that a LabView DAQ records. Early results in DI water show a decreasing parabolic force profile for the top bearing with increasing RPM while a decreasing linear force profile was present for the bottom bearing. For the adult full respiratory support application, the force profile shows a lifting of the impeller off the bottom bearing without any decoupling or decreased motor performance. Further testing will determine the stability of the impeller for the pediatric full respiratory support and adult low-flow CO2 removal applications.

Characterizing Deformation in Tissue Engineered Heart Valves under Dynamic Loading Conditions

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The correlation between mechanical loading conditions and cellular activity of heart valves is well-documented. Abnormal mechanical loading can lead to pathological cellular activity and a disruption in defined extracellular matrix (ECM) architecture. The converse of this concept can be applied to regenerative medicine, specifically by attempting to recreate physiological loading conditions of a tissue engineered heart valve (TEHV) to promote healthy tissue remodeling and ECM deposition. Our group has recently demonstrated a capacity for electrospinning elastomeric TEHVs with tailorable fibrillar micro-architecture by manipulating fabrication parameters such as the rotational and translational velocities of a grounded tri-leaflet mandrel. Utilizing this method, we hypothesized that it is possible to fabricate valves that successfully recreate mechanical properties of native heart valve tissue. In order to test this hypothesis, we placed TEHVs into a mock circulatory loop that reproduces physiological flow conditions and used a pair of high-speed cameras to capture images of a grid of markers on the valve leaflet. By stereoscopically calibrating the cameras, we were able to extract three-dimensional positions of the markers at defined pressure values. From the image sequence, we were able to measure both in-plane and out-of-plane deformations of the TEHV throughout the cardiac cycle. Comparing the deformation values to an unloaded condition, we were able to calculate a strain map across the surface of the valve leaflet with which it is possible to understand the mechanical response of our TEHVs compared to native tissue.

Effect of turbulent flow on damage to blood cells using the in vitro model of the assisted blood circulation

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Left ventricular assist devices (LVADs) are a viable option for the survival and improved quality of life for many patients with end-stage heart failure. However, this technology is often associated with complications such as mechanical damage to blood cells leading to hemolysis and thrombosis.
While blood flow in the vascular system is generally laminar, implantation of an LVAD device may introduce regions of non-physiological turbulent blood flow which has been proven to cause mechanical blood trauma. It has been previously demonstrated that higher levels of hemolysis were recorded in the presence of turbulent flow in the capillary tube at wall shear stresses ranging from 200 – 400 Pa while laminar flow at similar shear stresses did not generate similar hemolysis (Kameneva, et al., 2004). Since turbulent flow forces contribute to RBC damage, it is obvious that the same shear stresses may influence platelet function. Therefore, we proposed to compare effects of turbulent and laminar flows on platelet activation at similar shear stress conditions and may provide insight for thrombi occurrence in LVADs.

Ongoing experiments validating an in vitro setup by measuring hemolysis from turbulence at various shear stress conditions similar to the previous study, are currently underway. Ovine blood samples at identical hematocrit are prepared with various viscosities with the addition of Dextran-40 in order to produce either turbulent or laminar flow at the same shear stress conditions. These blood samples are driven at pre-determined pressure gradients through a small capillary tube where the presence of turbulent or laminar flow is confirmed by Reynolds number. The results of this study will show the effects of turbulence on injuries to blood cells via in vitro measurements of damage to RBCs (hemolysis) and platelets (activation), which are both clinically observed in heart-assist devices

Hand-held optical imaging for breast cancer therapy prediction

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Neoadjuvant chemotherapy is often used in breast cancer, shrinking the tumor and therefore permitting breast-conserving surgery, where mastectomy would otherwise be indicated. A pathologic complete response, as assessed after surgery, is associated with improved survival, but not all patients achieve this response. Prediction of treatment efficacy and patient outcome early in therapy is crucial for reducing morbidity and optimizing treatment regimes, but currently there is no accepted imaging modality for assessing treatment responses. Structural imaging modalities can track changes in tumor size, but can't reliably detect small residual disease. Optical imaging with near-infrared light is sensitive to tissue vascularization and monitoring of hemodynamic changes, and has shown promise for evaluating disease progression. Measurable changes have been found in baseline hemoglobin concentrations compared to healthy tissue, as well as in hemodynamic responses to perturbations, such as tissue compression. It has previously been established that in patients who achieve complete response to therapy, the hemodynamic reaction of the tumor tends to normalize over the course of therapy and can therefore be used as marker of disease state. We are building off of such findings and have developed an inexpensive, handheld, optical imaging device to non-invasively measure the response of breast tumors to compression. Force of compression and optical images are obtained concurrently during the sustained application and then slow release of pressure. The resulting hemodynamic response (such as blanching of tissue followed by re-perfusion) will be compared between tumor and healthy breast. Experiments in breast tissue-mimicking phantoms show that compression of tissue enhances the optical contrast and detectability of deep situated inclusions. We will present on the design of the hand-held device, phantom studies, as well as preliminary data on healthy volunteers.

Prevention of Ischemia-Reperfusion Injury and Chronic Rejection in a Porcine Vascularized Composite Allotransplantation Model

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Background: Restoration of catastrophic extremity and maxillofacial trauma utilizing vascularized composite allotransplantation (VCA) is superior to conventional reconstruction, but systemic immunosuppression and chronic rejection (CR) limit application. Technology to reduce immunosuppression load and diminish CR would have a substantial impact. To mitigate ischemia-reperfusion injury (IRI) and the subsequent CR, we evaluate the efficacy of ex-vivo tissue preservation using two novel techniques: (1) sub-normothermia (21°C) machine perfusion/hemoglobin-based oxygen carrier (MP/HBOC) and (2) normothermia (37°C) hyperbaric oxygenated perfusion/Kidney Preservation Solution (HOT/KPS) for 24 hours.

Methods: A proven porcine myocutaneous heterotopic transplant flap model was performed. Controls (n=16) underwent cold storage preservation (4°C) with University of Wisconsin solution (CSP/UW) for 5 hours prior to transplant. Experimental group 1 (n=16) underwent MP/HBOC for 24 hours at 21°C while group 2 (n=8) underwent HOT/KPS for 24 hours at 37°C. Biopsies for evidence of viability and blood samples to evaluate markers of IRI were obtained until designated end points (24 hours ex-vivo & 14 days in-vivo).

Results: All transplanted tissue remained clinically viable at the 14-day end-point. Histological evaluation of controls revealed sporadic mild to moderate (Grade II/III) necrosis per Banff classification. Experimental flaps placed in HOT/KPS preliminarily indicate decreased histologic evidence of injury and necrosis ranging from none to mild (Grade 0/I). Serum analysis evaluating markers of IRI were obtained with evidence of attenuation for both experimental techniques.

Conclusion: Preservation of a VCA for >24 hours while minimizing IRI, thereby mitigating CR, will have a profound clinical impact in VCA and solid organ transplantation. Based on the preliminary data, we believe efficient tissue oxygenation promoted by sub-normothermic MP/HBOC and normothermic HOT/KPS in VCA could (1) extend graft preservation times and improve donor access across geographic spans, (2) enable increased efficacy of ex-vivo targeted graft treatment, and (3) optimize tissue viability prior to transplantation.

Intracortical neural stimulation with untethered, ultrasmall carbon fiber electrodes mediated by the photoelectric effect

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Neural stimulation with tethered, electrically activated probes is damaging to neural tissue and lacks good spatial selectivity and stable chronic performance. The photoelectric effect, which converts incident light into electric potential and heat, provides an opportunity for a tetherless stimulation method. We propose a novel stimulation paradigm that relies on the photoelectric effect to stimulate neurons around a free-floating, ultrasmall (7-8μm diameter) carbon fiber probe. A 2-photon microscope induced photo-stimulation with a near-infrared (NIR) laser. Directing NIR at the implanted probe causes a local cathodic potential pulse with minimal leakage current. Chronoamperometry and chronopotentiometry were used to characterize the electrochemical properties of photo-stimulation, while the fluorescence of Rhodamine-B was used to quantify temperature changes. Photo-stimulation caused a local cathodic potential pulse with minimal leakage current. Stimulation induced voltage deflections of 0.05 - 0.4V in vitro, varying linearly with the power of the laser source (5 – 40 mW). Temperature increases in the immediate vicinity of the electrode were limited to 2.5°C, suggesting that this stimulation modality can be used without inducing heat damage. Successful stimulation was supported in vivo by increased calcium fluorescence in local neurons at stimulation onset in a transgenic GCaMP-3 mouse model. Furthermore, cells activated by photo-stimulation were closer to the electrode than with electrical stimulation under similar conditions, indicating increased spatial precision. Our results support the hypothesis that the proposed photoelectric method for neural stimulation is effective.

Theranostic Analgesic Regenerative Gel-Emulsion Technology (T.A.R.G.E.T.) Platform for Local Analgesia and Promotion of Nerve Regeneration

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Background: Acute neuropathic pain (ANP) management remains a significant therapeutic challenge. The TARGET platform is a biocompatible, thermoresponsive nanoemulgel, laden with NIRF-labeled theranostic nanoemulsions, designed for macrophage-targeted drug delivery of COX-2 inhibitors. This integration of dual therapeutic and diagnostic functions in a single local delivery platform provides an imaging signature of the underlying pathology, delivers a therapeutic agent (COX-2 inhibitor), and allows for monitoring drug delivery and therapeutic efficacy by live NIRF imaging. The TARGET platform represents a theranostic nanoemulsion gel which incorporates a triphasic perfluoropolyether (PFPE) nanoemulsion into a thermoresponsive hydrogel matrix. The nanoemulgel material is designed to flow at temperatures up to 90 degrees F while gelling at body temperature to facilitate extended drug release for up to 30 days.

Methods: Presented platform is formulated as a thermoresponsive biocompatible hydrogel matrix loaded with nanoemulsions (NEs, 100-130nm) for local immunomodulation and local analgesia. The distinct near-infrared fluorescence (NIRF) imaging signatures incorporated into nanosystems provide unbiased measures of where specific non-opioid drugs are delivered as well as how long they are present in specific target cells. Pain behavior studies and NIRF whole body and ex vivo imaging are utilized to evaluate TARGET in rats (naïve and injured).

Results: We present fabrication and optimization in vitro of nanoemulsions and nanoemulsion-loaded hydrogel platforms with selected non-opioid drug payloads (anti-inflammatory and analgesic drugs). We present NIRF imaging and preliminary behaviour results upon administration of TARGET nanoemulsions via tail vein injection in naïve and injured rats.

Conclusion: To the best of our knowledge this is the first example of targeted theranostic (therapeutic and diagnostic) nanomedicine for local analgesia. Our preliminary results demonstrate adequate bioavailability of the TARGET nanoemulsion administered via tail vein, and accumulation at the site of injury. Further, we show that the TARGET platform can be fully optimized for local analgesia.

Ultrasound-targeted microbubble cavitation with sodium nitrite synergistically enhances nitric oxide production and microvascular perfusion

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Congestive heart failure following AMI is rising due to microvascular obstruction (MVO), a combination of obstruction by atherothrombotic debris and inflammation accentuated by decreased nitric oxide bioavailability. We previously demonstrated that ultrasound-targeted microbubble cavitation (UTMC) may relieve MVO and increase endogenous NO production.

We hypothesized that co-administration of nitrite, a source of exogenous NO, would enhance the therapeutic effects of UTMC through increasing NO bioavailability and microvascular perfusion.

UTMC was delivered to a rat hindlimb using lipid microbubbles sonicated by therapeutic ultrasound pulses for 2 minutes. In select groups, sodium nitrite and/or N-Nitro-L-arginine methyl ester (L-NAME), an eNOS inhibitor, were given 5 minutes prior to UTMC. An intramuscular NO probe was placed for real-time concentration measurements to 30 minutes post-UTMC. Microvascular blood volume was measured using contrast ultrasound with infusion of Definity microbubbles at baseline, 3, 6, 10, and 30 minutes post-UTMC. Treatment effects were evaluated using a mixed effect model with a Bonferroni correction.

Treatment factors of UTMC, nitrite, and L-NAME had significant interactions. UTMC and nitrite together showed significantly increased blood volumes at all observation points compared to baseline and UTMC alone. Addition of LNAME to UTMC and nitrite resulted in decreased blood volume at 3, 10, and 30 minutes below baseline. Similar results were seen in NO concentration, where UTMC and nitrite resulted in significant increases above either UTMC or nitrite alone that were ablated with LNAME.

We have shown that UTMC and nitrite therapy results in synergistic improvements of microvascular blood volume and NO concentration that are partially dependent on eNOS activity. These results provide mechanistic insight into UTMC and means of enhancing its efficacy through increasing NO bioavailability.

Bioengeneered the Cordae Tendineae apparatus

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Objective: Current methods for Chordae Tendineae (CT) replacement have demonstrated adequate midterm clinical results; however, the materials utilized do not recapitulate the microarchitecture nor the mechanics of the native CT and most importantly they do not promote tissue regeneration. This study introduces a novel mandrel-less electrospinning approach to create a Biomimetic Engineered CT (BECT) using bioabsorbable polymers.

Methods: Fresh porcine CT (PCT) were harvested and divided in 6 categories: Anterior Marginal (AM), Anterior Strut (AS), Commissural (CM), Posterior Basal (PB), Posterior Intermediate (PI) and Posterior Marginal (PM). Scanning Electron Microscopy (SEM), Uniaxial Tensile Testing (UTT) and histology evaluation coupled with digital image analysis have been utilize to evaluate PCT, structure, function and cell distribution. Poly(ester Urethane) Urea (PEUU) was used to fabricate BECT via a mandrel-less electrospinning method. BECT structure and mechanics were compared to PCT. BECT were seeded with NIH/3T3 fibroblasts and cultured. Cell infiltration and proliferation were evaluated via histology at day 1, 3 and 7.

Results: PCT histology showed smaller cell density in AS CT vs PM CT (832 vs 1646 cells/mm2, $p<0.0211$). UTT demonstrated stiffer marginal CT than strut CT (E= 245 vs 68.93 MPa). This was consistent with the level of collagen fiber alignment measured in marginal and strut CT. BECT produced via mandrel-less deposition recapitulated PCT shape, diameter, and length. SEM analysis showed highly aligned fiber microstructure comparable with PCT. Unseeded BECT reported a lower elastic modulus than the PCT. Histology of cell-seeded BECT demonstrated cell adhesion and initial infiltration in 7 days of static culture.

Conclusion: A novel mandrel-less electrospinning method to process bioinspired BECT has been introduced. We demonstrated adequate scaffold cellular adhesion, proliferation and the ability to recapitulate CT native structure. BECT demonstrate potential to be translated to a more robust and longer-functioning strategy for CT replacement in valve surger

Mechanisms of extracellular matrix (ECM) hydrogel biodegradation: an in vitro assay

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Currently, the only widely available treatment for stroke is physical therapy. Cell therapy is an emergent avenue, but is still in clinical trials. Neither of these therapies are completely successful, and they do not revert brain tissue loss. Provision of a bioscaffold, consisting of ECM hydrogels, to promote invasion of neural cells into the stroke cavity may be necessary to regenerate lost tissue. Degradation of these hydrogels is, however, required to ensure tissue regeneration. Matrix metalloproteinases (MMPs), are endogenous enzymes that degrade extracellular proteins, such as collagen, which is the main component in these hydrogels. By adding these MMPs on a hydrogel in vitro, there will be a better understanding of the mechanism of the degradation that occurs when gels are injected and degraded in vivo. This study will focus on adding these MMPs to the hydrogels in order to define their role in biodegradation using an in vitro assay. To accomplish this goal, a dose response of MMPs will be evaluated against different concentrations of hydrogels. Gel weight changes over a time period of 14 days will determine hydrolysis of the gels in the absence of MMPs. The supernatant will be collected daily and replaced with a fresh MMP solution. The supernatant will be analyzed using Western Blots to determine if the collagen is actually degraded (degraded collagen does not have the same band size as intact collagen) and ELISAs to determine growth factor release. Preliminary studies indicate that addition of water alone will hydrolyze the gels and reduce their weight over 14 days. Understanding the influence of MMPs on hydrogel degradation will be essential to improve the design of bioscaffolds and control the process of tissue regeneration.

Design and Characterization of a Compliance-matched Biopolymer Tissue-Engineered Vascular Graft

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Coronary artery bypass grafting (CABG) is comply used to treat coronary artery disease with nearly 400,000 bypass procedures performed annually in North America. Autologous tissue is not always available for bypass and synthetic grafts (Dacron, ePTFE) have a high failure rate in small diameter applications in part due to compliance mismatch. Our research team is therefore developing a computationally and experimentally optimized tissue-engineered vascular graft whose compliance can be tuned for optimum performance in CABG. In addition to tuning for mechanical performance, we are also developing an approach to endothelialize our construct and promote its healthy remodeling and integration in-vivo. Future work in this project will also assess the vasoreactivity of our vascular graft.

The Therapeutically Induced Matrisome (Tim) Protects and Promotes Metastatic Disease

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The lethal progression of melanoma is enabled by refractory micrometastases. These small tumors seed ectopic tissues wherein the new microenvironment drives cancer cell dormancy, whose populations are immune-silent and fail to respond to chemotherapy in contrast to primary lesions. Currently, there remains a paucity of information surrounding metastatic survival and the emergence of drug resistant niches. Knowledge of such events has been largely limited by current model systems that cannot isolate these individual cells and small nodules. Thus a novel ex vivo microphysiological systems (MPS) model of human metastatic melanoma has been developed to elucidate mechanisms for melanoma survival throughout progression. Preliminary evidence using ex vivo human skin organ cultures correlates with pathophysiological changes in primary melanomas, particularly the extracellular matrix proteins in the skin. Proteomic analysis identified a marked increase in the wound response protein Tenascin-C (TNC), which was shown to be absent in quiescent tissue. These findings were corroborated using expression data from The Cancer Genome Atlas and Human Proteome Project, both showing TNC upregulation in several carcinomas. Immunohistofluorescent staining for TNC in human melanoma metastasis to lung shows enrichment of TNC in the metastatic site. Here, TNC has been shown to increase with the malignant mesenchymal phenotype, while epithelial marker of quiescence E-Cadherin (E-cad) is downregulated. Western blot identification of melanomas shows an epithelial E-CadHI shift to E-CadLO and TNC/EGFRHI during both initial metastasis with this being reversed upon metastatic dormancy. Small molecule inhibitors for EGFR signaling are being evaluated to reverse the pro-metastatic ECM phenotype to halt disease.

Visualizing the microvasculature of the optic nerve head and their changes during intraocular pressure increases

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Introduction: There is a clinical association between nerve fiber loss in glaucoma and systemic microvasculature abnormalities. However, almost nothing is known about microvasculature morphology and topology within the optic nerve head, through which the nerves transmitting visual information to the brain pass, or how the vasculature is affected by changes in intraocular pressure (IOP). Our goal was to visualize the optic nerve head microvessels and determine their deformations under acutely elevated IOP.

Methods: Three pig heads, within 8 hours of death, were perfused through the external ophthalmic artery with PBS to remove clots then with a fluorescent dye to label the blood vessels in the eye. The posterior pole of each eye was then mounted in a custom-made inflation chamber for control of IOP with simultaneous imaging. Images of collagen beams and capillaries were acquired using structured light illumination-enhanced microscopy at IOPs of 15, 25 and 40 mmHg (normal, high and very high, respectively). Microvessel tortuosity was measured from the images and paired two-sample t-tests used to test for significant changes with elevated IOP.

Results: Microvessels were highly tortuous at 15 mmHg (up to 1.45). In all but one eye, tortuosity decreased significantly as IOP increased from 15 to 25 mmHg ($p<0.01$), and tortuosity decreased significantly in every eye as IOP increased from 15 to 40 mmHg ($p<0.01$). In a few microvessels, tortuosity increased with IOP.

Discussion: Overall, tortuosity decreased as IOP increased, but some microvessel segments did become more tortuous. While tortuosity in microvessels is often regarded as problematic because it may reduce blood flow, in the optic nerve head the higher tortuosity at 15 mmHg may provide “slack” that protects the microvessels from overstretch damage under elevated IOP. We speculate that low tortuosity microvessels could be a risk factor for damage under high IOP.

The Development of Ovarian Hydrogels as an Alternative Strategy for Fertility Preservation

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Female cancer patients have a significant risk of losing reproductive function due to cytotoxic treatments. The current options for fertility preservation are limited to (1) re-implantation of cryopreserved ovarian tissues or (2) follicle isolation for in vitro maturation (IVM). To date, there has been an increasing number of successful orthotopic transplantation procedures, resulting in greater than 130 live-births; however, the efficiency of this method remains low, with live-birth rates ranging from 23-36%. To improve these outcomes, we have developed a bioactive tissue-specific hydrogel from decellularized porcine ovarian tissues, which could provide an alternative biomaterial to support follicle maturation.

Porcine ovaries were decellularized using a series of detergents then characterized using histological and biochemical techniques. Decellularized tissues were lyophilized, milled, enzymatically digested and neutralized to form hydrogels under physiologic conditions. Hydrogel mechanical properties and ultrastructure were assessed using rheology and scanning electron microscopy (SEM). Secondary ovarian follicles were isolated from young female mice and encapsulated in ovarian hydrogel (OECM) droplets then cultured for 12 days in vitro. In addition, a pool of follicles were microinjected into mouse ovaries using OECM to assess the feasibility of in vivo follicle delivery.

Ovarian tissues were effectively decellularized with a 98.4% removal of dsDNA. Ovarian-specific hormones, growth factors, and ECM proteins were also preserved after decellularization. The OECM hydrogel storage and loss modulus increased in response to higher ECM concentration and SEM analysis demonstrated a highly porous and fibrous ultrastructure. Ovarian follicles encapsulated in OECM droplets reached metaphase II, which was confirmed by the presence of a polar body and follicles successfully co-localized with the OECM post in vivo injection. Our results indicate that OECM hydrogels can support IVM to obtain mature oocytes for fertilization and may be utilized as a delivery vehicle for in vivo follicle transplant providing a potential therapy for restoring reproductive function.

In Vitro Differentiation of BMSCs in PGH and Gelatin Hydrogels

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Temporomandibular joint disorders (TMDs) impact about 10 million Americans (1). This imposes a tremendous financial burden on society in the form of health care costs, disability and loss of productivity as this affects the quality of life for those who suffer from them. Symptoms of TMDs include joint pain, limited mouth opening, deviation of the jaw, locking, dislocation, and pain in the masticatory muscles during jaw movement. Currently one of the solutions for advanced, downstream degeneration of the TMJ are prosthetic devices; however, these can host many other problems. This study aims to present a tissue engineering alternative to these current methods by studying the use of hydrogels for the regeneration of both the mandibular cartilage and the subchondral bone.

This study's goal was to test the efficacy of using gelatin and a PGH (P: poly ethylene glycol, G: gelatin, H: heparin) hydrogel scaffolds seeded with goat bone marrow stem cells and allow them to differentiate into cartilage and bone. This was done by seeding the cells into cross-linkable hydrogels and culturing them in both chondrogenic and osteogenic media for 6 weeks. The experimental groups tested were: 1) gelatin hydrogels cultured in osteogenic media, 2) gelatin hydrogels cultured in chondrogenic media, 3) PGH hydrogels cultured in osteogenic media, 4) PGH hydrogels cultured in chondrogenic media. Histological staining for glycosaminoglycans (GAGs) and phosphates were performed and a biochemical content assay for GAG were performed on the scaffolds after their 6 week time points.

From the histology, both the gelatin and PGH hydrogels promoted GAG production, chondrocyte differentiation, and inhibited mineralization within the scaffold after 6 weeks of culture in chondrogenic media. The gelatin group demonstrated phosphate presence when cultured in osteogenic media after 6 weeks. However, the PGH group cultured in osteogenic media, did not show any phosphate present in the Von Kossa stain. These results show that PGH shows promise in being able to promote chondrogenesis and prevent mineralization, whereas gelatin can be used to promote mineralization.

MBV-associated IL-33 Protects Against Chronic Heart Transplant Rejection

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Chronic rejection following heart transplantation is associated with cardiac allograft vasculopathy and myocardial fibrosis, which result in the failure of more than 50% of transplants within 11 years. The pathophysiology of the fibrotic change is thought to be the result of chronic low-grade immune-mediated vasculitis. The pro-inflammatory macrophage and T-cell phenotype can be mitigated by anti-inflammatory and immunosuppressive drugs, but these are ineffective in preventing pathogenic remodeling. An alternative immunomodulatory, but not immunosuppressive, approach involves signaling molecules contained within the extracellular matrix. Matrix bound nanovesicles (MBV) embedded within the extracellular matrix contain biologically active molecules that can rapidly and directly activate macrophages to a pro-remodeling phenotype.

MBV are a rich and stable source of extra-nuclear interleukin-33 (IL-33). IL-33 is an IL-1 family cytokine that is classically considered an alarmin but has emerging reparative and immunoregulatory properties. We have found that the IL-33 encapsulated within the MBV can bypass the classical IL-33/ST2 receptor signaling pathway and is required to activate macrophages toward a reparative M2-like phenotype via a non-canonical ST2-independent pathway. In mouse model of heart transplant, the absence of IL-33 in grafts isolated from il33-/- mice resulted in increased chronic rejection-associated fibrosis, vasculopathy and immune cell infiltration in both wild-type and il33-/- recipients. Administration of a collagen hydrogel loaded with IL-33+ MBV to IL-33 deficient grafts immediately following transplantation limited the infiltration of pro-inflammatory immune cells and decreased the extent of fibrosis and vascular occlusion. These data suggest that IL -33 encapsulated within the MBV promotes a reparative phenotype in infiltrating immune cells and helps to prevent allograft fibrotic diseases. An MBV-based approach may represent an alternative immunomodulatory therapy to protect against chronic rejection of heart transplants.

Growth & differentiation of human bronchial epithelial cells on decellularized trachea ECM scaffold for airway replacement

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Tracheal injury leads to significant and often life-threatening complications. Limited treatment options exist and include resection of the damaged trachea with attempts at reconstruction. Tracheal replacement with the use of synthetic constructs results in chronic inflammation and subsequent fibrosis and stricture. Recent reports using decellularized mammalian tracheas suggest a potentially life-saving therapeutic alternative. These 3-dimensional extracellular matrix (ECM)-based biologic scaffolds, seeded with autologous cells, can remodel when placed in-situ and integrate with surrounding tissue to replace the structure and function of the native trachea. Our goal is to engineer a tracheal segment for transplantation using decellularized tracheas seeded with airway epithelial cells.

In the present study, porcine tracheas were decellularized chemically and mechanically with the use of detergent and enzymatic solutions and a negative pressure chamber. Decellularization was verified by histologic staining and analysis of residual DNA content. Primary human bronchial epithelial cells (HBEC) were seeded directly on the decellularized tracheal scaffolds, and upon hydrogels made from solubilized tracheal ECM. Hydrogels have been used in a number of clinical applications and their ease of delivery and manipulation make them an excellent candidate as a substrate lining for tracheal bioscaffolds. Growth and differentiation were assessed histologically by H&E, alcian blue and acetylated tubulin.

Results show that HBEC are able to proliferate and differentiate on both the intact decellularized tracheal scaffold and the tracheal hydrogels.

Current studies involve implantation of the decellularized scaffold in vivoto determine immune response, host cell infiltration, epithelial differentiation, vascularization, and function. These orthotopically implanted tracheal bioscaffolds can be cell-seeded either prior to in vivo placement or in situ. Results of in vivo studies are pending.

Controlled Silk Degradation Using Non-Invasive Ultrasound for Tissue Regeneration

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Often biomaterials for tissue regeneration are developed with set mechanical properties and degradation profiles. However, a patient's capacity for tissue regeneration varies based on age, nutritional status, disease state, lifestyle, and gender. There is currently no strategy to account for this variability in tissue regeneration. Silk fibroin is a strong candidate for use in patient-specific tissue regeneration due to its tunable degradation rates, mechanical properties, and ability to be conjugated with growth factors and alternative therapeutics to enhance regeneration. Clinically, ultrasound is mostly used for imaging and diagnostic applications, but it is rapidly gaining attention for therapeutic applications. It has also been shown that therapeutic levels of ultrasound decrease the molecular weight of silk. However, no studies have looked at using ultrasound to monitor and control silk scaffold degradation. To control tissue remodeling and scaffold degradation to maintain intrinsic mechanical properties through the healing process, diagnostic and therapeutic ultrasound will be paired to monitor and induce degradation, respectively. This will create a patient-specific biomaterial approach for those in need of tissue or organ replacements. To date, our research shows that silk scaffolds experience a weight reduction when exposed to ultrasound. This occurs without a change in crystallinity, indicating that the crystalline portions of silk are unaffected. Models will be fit to degradation data to predict the proper ultrasound exposure time for each patient. In the future, we envision this approach being used to grow mechanically stable, functional tissues for all patients.

Adventitial Extracellular Matrix Hydrogel Improves Sprouting of Human Thoracic Aortic Aneurysm-Derived Pericytes

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Introduction: Adventitial extracellular matrix bioscaffolds exert growth factor-mediated bioactivity making it an attractive biomaterial for disease modeling and therapeutic opportunities for thoracic aortic aneurysm. Our prior work uncovered down-regulation of several pro-angiogenic factors in the adventitia from aneurysmal aortic specimens and reduced vasa vasorum density. We classified several populations of vasa vasorum-associated cells including pericytes that were found to sprout from spontaneously formed spheroids when cultured on Matrigel substrates. In this study, we investigated the influence of adventitial ECM on vasa vasorum-derived pericyte vasculogenic function. We hypothesized that aneurysm-derived pericytes exhibit reduced sprouting and addition of normal adventitial ECM restores sprouting through an FGF-mediated pathway.

Methods and Materials: Human ascending aortic adventitia specimens were collected from patients undergoing ascending aortic and/or aortic valve replacement, or heart transplantation with Institutional Review Board approval and using an informed consent process. Human (n=40) adventitial specimens were decellularized, processed to lyophilized powder and pepsin digested as previously described to prepare adventitial extracellular matrix bioscaffolds (AdvECMs). Adventitial pericytes were isolated from normal, bicuspid aortic valve associated aneurysmal, and degenerative aneurysmal human aorta (n=3) as previously described and culture expanded. Pericytes were allowed to spontaneously form spheroids on growth factor reduced Matrigel substrate in the presence or absence of normal or diseased AdvECMs (0-250 μg/mL) and the FGFR inhibitor PD173074 (100 nM) or DMSO as the vehicle control. Total sprout length from pericyte spheroids was quantified from phase contrast images over 96 hr. Statistical analysis was carried out utilizing one-way ANOVA.

Results: Pericytes derived from bicuspid aortic valve- associated aneurysmal aorta and specimens of degenerative aneurysm exhibited decreased sprouting when compared to normal pericytes (107 ± 22 vs 1207 ± 342, $p < 0.001$; 377 ± 130 vs 1207 ± 342, $p < 0.001$, respectively). Addition of normal aorta-derived hAdvECM increased total length of sprouts extending from pericyte spheroids when compared with pepsin controls (1608 ± 312 vs 984 ± 184, $p=0.028$) while addition of degenerative aneurysmal hAdvECM exhibited no significant difference compared to pepsin (1251 ± 217 vs 984 ± 184, $p=0.343$). Addition of PD173074 decreased sprouting when compared with vehicle control and normal hAdvECM (531 ± 132 vs 1452 ± 264, $p < 0.001$; 531 ± 132 vs 1608 ± 312, $p < 0.001$, respectively).

Conclusions: Decreased sprouting in aneurysm-derived pericytes demonstrates that aberrations in pericyte function may adversely affect vasa vasorum in aneurysmal tissues. Normal hAdvECM-induced sprouting and PD173074-mediated inhibition of sprouting suggests that adventitial ECM promotes vasculogenic activity via an FGF-mediated pathway. Restoration of pericyte sprouting within hAdvECM-containing scaffolds supports the use of AdvECM as a potential therapeutic biomaterial for microvascular regeneration in human aortic disease.

Compliance Manipulation of Polycaprolactone/Gelatin Tissue Engineered Vascular Grafts in a Rat Model

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In the U.S., Coronary artery bypass surgery occurs in over 400,000 patients with a one year patency rate ranging from 60%-95% [1, 2]. This lack of patency is attributed to graft thrombosis and/or intimal hyperplasia [3, 4]. While there are many factors that contribute to procedural failure, graft compliance mismatch between the implant and the native vessel is thought to contribute to the loss of patency [3, 5]. The Soft Tissue and Biomechanics Laboratory has demonstrated that the compliance of a vascular graft can be modulated using a computational and experimental approach [6]. In this study, the composition of a vascular graft will be modulated to illustrate the role compliance facilitates in vascular graft design using a rat model.

The compliance of rat aorta will be measured using microbiaxial optomechanical device. An acellular vascular graft with varying ratios of gelatin and polycaprolactone will be fabricated using electrospinning. These grafts will be compliance matched (CM) or hypocompliant to native rat aorta, crosslinked in a genipin solution, and implanted interpositionally into the aorta of a Sprague Dawley rat (n=7). While the graft is implanted, doppler ultrasound will measure in vivo average blood velocity and aorta diameter at 1, 2, and 4 weeks. After one month, the rat will be sacrificed and evaluated for infiltration of vascular smooth muscle cells, endothelium, macrophages, and vascular dimensions.

The compliance of rat aorta, CM graft, and hypocompliant graft were 5±5.6x10-4, 5±0.49x10-4, and 2.5±2x10-4 mmHg-1, respectively. In vivo diameter measurements showed that the CM group was similar to native aorta while the hypocompliant graft became more compliant over the duration of the study. The in vivo average blood velocity between the experimental groups was not found to be significant. At 14 and 28 days the hypocompliant and CM were significantly slower than native blood flow, respectively. Cellular infiltration and assessment is still ongoing.

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Age-related compositional and biomechanical changes in the cardiac extracellular matrix promote altered macrophage phenotype and function

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Recent work has identified several developmentally, phenotypically, and functionally distinct macrophage subsets within the mammalian cardiovascular system. These subsets are differentially regulated with increasing age, however the mechanisms driving this shift remain to be elucidated. As the cardiovascular system is known to undergo numerous compositional and biomechanical alterations with increasing age, we hypothesize that these age-related alterations in the cardiac microenvironment drive altered the observed altered phenotype and functionality in naïve macrophage populations which occurs with increasing age. To understand the impact of altered cardiac extracellular matrix (ECM) composition on macrophage populations, naïve bone marrow-derived macrophages were exposed to either young or aged decellularized cardiac ECM degradation products coupled with subsequent pro- or anti-inflammatory cytokine exposure. The impacts of biomechanical alterations on naïve macrophages were assessed through macrophage culture on functionalized polydimethylsiloxane gels of varying stiffnesses which were either not coated, coated with young cardiac ECM, or coated with aged cardiac ECM. Exposure to aged cardiac extracellular matrix degradation products induced increased nitric oxide production both at baseline and following exposure to both pro- and anti-inflammatory cytokines. Aged cardiac ECM exposure additionally induced attenuated gene expression levels following both pro- and anti-inflammatory cytokine exposure. These results indicate that naïve macrophages exposed to aged cardiac ECM degradation products, such as those they would encounter upon extravasating into aged tissue sites, promote attenuated gene expression following cytokine stimulation coupled with altered regulation of radical oxidant secretion. Additionally, macrophages exhibited altered cell morphology, phenotype, and function when cultured on gels of varying stiffness. These results suggest the stiffening of cardiovascular tissue which occurs with physiological aging can promote altered regulation of naïve macrophage phenotype and function. As bone marrow-derived macrophages comprise an increasingly large percentage of the total macrophage pool with aging, the results derived from this study will serve to help elucidate potential mechanisms underlying the macrophage-mediated contributions to the age-correlated acquisition of cardiovascular disease pathologies.

Creating innervated vascularized muscle flaps from elastic, cellularized biocomposites developed in situ for facial muscle reconstruction

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Reanimation of the face is still challenging due to its intricate element comprised of underlying bones and cartilages that support muscles, subcutaneous and skin tissues. Though free flap transfer is one of the reconstructive methods, it often induces aesthetic insufficiency due to much volume of the current muscular flap. In the previous study we identified the dermal ECM/PEUU composite scaffold micro-integrated with MDSCs as the most favorable for tissue regeneration. We assessed clinical efficacy and safety of the scaffold in two animal models. To assess flap transferability, the scaffold was implanted on the adductor muscle and the femoral artery, vein and the stump of the femoral nerve in rat model. At the 8 weeks and 16 weeks, the proto-flap was transferred to the contralateral defect prepared in the adductor magnus including microvascular anastomosis and neurorrhaphy. In vivo muscle contraction test was performed 16 weeks after the flap transfer. Flaps were harvested and histologically assessed right after the muscle test. No statistically significant difference was observed between the study groups and the controls at both 8 and 16 weeks.The Scutuloauricularis muscle of rabbit was utilized to evaluate and visualize facial paralysis. Following the result of rat model, the proto-flap was transferred to the face where facial paralysis was created by transecting the auricularis rostalis nerve and by removing the scutuloauricularis muscle 8 weeks after the implantation of the scaffold. Rabbit study is now ongoing. This result suggests that the generated muscle flap would be utilized for performing functional reconstruction which requires various shape of muscle such as in face, neck and hand.

Hypercapnia exposes deficiencies in cerebrovascular response and tissue oxygenation of transgenic AD mice

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Background: The brain has no significant energy storage, and neuronal activity is tightly coupled with oxygen consumption and blood flow. This phenomenon is so widely accepted that the vascular deficits during Alzheimer's disease (AD) progression were considered a byproduct of the impaired neuronal activity. Growing evidence points at vascular dysfunction as an independent contributor to the AD etiology. Teasing apart coupled events presents a challenge for determining their separate contributions. Breathing hypercapnic gas raises the carbon dioxide (CO2) level in blood. This evokes a robust systemic vascular response that increases cerebral blood flow in the brain without altering neuronal activity. In the present study, we used hypercapnia to evaluate the cerebrovascular health, vessel reactivity and tissue oxygenation of transgenic AD mice.

Methods: Hypercapnia was induced by transiently changing the inspired gas from air to air containing 10% CO2 for 180 sec. Laser Doppler probe and Clark-type oxygen sensors were used to measure simultaneously blood flow and partial oxygen tension in the mouse cortex. Hemoglobin-based optical intrinsic signal was concurrently acquired to evaluate blood volume and vascular diameter. We also estimated the vascular and tissue plaque load.

Results: AD mice had significantly lower blood flow response and tissue oxygenation than controls, exposing deficiencies in cerebrovascular response and oxygen metabolism. The baseline and changes in the vascular diameter measured on the hemoglobin-based images were lower in AD animals. We found a rapid drop in the oxygen diffusion gradient between arteries and cortical tissues compared to age-matched controls.

Conclusions: Cerebrovascular deficiencies in AD mice appear to be independent from decreased neuronal activity. Our findings have a direct translation in human breath-holding functional imaging lending itself as a possible early diagnostic test, therapeutic target and a tool to evaluate AD treatments.

A Novel Mouse Model for Corneal Scarring

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Purpose: Corneal opacity caused by fibrotic scar tissue accumulates in response to corneal inflammatory response and remains long-term in the stroma, often correctable only by surgery. Numerous recent studies have investigated innovative approaches to reversal of corneal scarring using non-surgical means, however, existing mouse models limit these studies due to the optical properties the mouse cornea which exhibits little opacity in response to fibrotic matrix accumulation. The current study investigates the hypothesis that corneal scaring can be modeled using a transgenic mouse in which enhanced green fluorescent protein (EGFP) is expressed by a collagen 3 promoter.

Methods: The transgenic line Tg(Col3a1-EGFP) DJ124Gsat (Col3-EGFP) was obtained from MGI via Mouse Biology Program at UC Davis. The animals were bred and used as heterozygotes, used experimentally at 6-8 weeks. Debridement and alkali wounding of corneas were carried out using published protocols under ARVO guidelines and approved IACUC protocols. Corneal fluorescence in vivo was examined using Olympus dissecting microscope. Ex vivo, corneas were examined using standard fluorescence microscopy, histology, microplate fluorimeter, ELISA for EGFP and by qPCR. Image analysis used Nikon NIS Elements software. Statistical significance was determined by t-test and ANOVA.

Results: Col3-EGFP mice are healthy, vigorous, and breed with large litters showing no morphological or behavioral abnormalities. After physical or chemical wounding, corneal opacity was observed at 3 days, but corneal EGFP expression was not observed until 7 days, peaking at 10-14 days and still detected at 28 days. EGFP could be readily detected by in vivo imaging. Ex vivo, EGFP was observed in stromal cells peripheral to the wound and could be quantified by counting individual cells, or by ELISA for GFP in corneal extracts. qPCR showed EGFP mRNA to be upregulated synchronously with mRNA for Col3a1 and smooth muscle actin (Acta2).

Conclusions: Mouse corneal opacity in response to wounding does not provide an accurate measure of stromal fibrosis, a prime component of long-lasting human corneal scars. The Col3-EGFP mouse provides a new tool to detect corneal scarring in real-time and will be useful in modeling the ability of stem cells and other innovative treatments in preventing and reversing corneal blindness.

Robust Bone Formation through the Developmental Condensation and Endochondral Ossification of human Mesenchymal Stem Cells within their Own Extracellular Matrix

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Large bone defects, pathological fractures and aging often result in delayed bone healing or nonunion. Autologous bone grafting has been frequently used to treat nonunion, but it still has limits, including donor site morbidity, limited tissue availability, and molding challenges. Mesenchymal stem cells (MSCs)-based bone-tissue engineering offers exciting promise to overcome the obstacles of using native tissue. In respect to the developmental endochondral ossification (ECO), there is an increasing interest to induce MSCs into hypertrophic chondrocytes (cartilage) first and then remodel them into bone tissue. Recently, we have developed a scaffold-free approach to generated cartilage from MSCs. In this novel method, MSCs were impregnated within their own extracellular matrix (MSC-mECM) and underwent robust developmental condensation and chondrogenesis upon chondroinduction. In this study, we tested the utility of MSCs-mECM for bone regeneration both in vitro and in vivo.

MSCs were cultured to reach hyper-confluency, then cultured for additional 10 days with the supplementation of L-ascorbic acid, which allowed the deposit of copious ECM. After 5 minutes of trypsin-EDTA treatment, 3D MSC-derived ECM constructs were formed and maintained in conventional osteogenic (OM, control group) or chondrogenic (CM, ECO group) medium respectively. After 4 weeks of culturing, both groups were cultured in osteogenic medium for another 4 weeks or implanted in SCID mice for in vivo bone formation.

As our results showed, After 4 weeks culture in CM, MSC-mECM not only deposited cartilage matrix, but also displayed significant enhanced expression of ALP, suggesting the formation of hypertrophic cartilage. After another 4 weeks culture in OM, constructs in ECO group showed more production of osteocalcin (OCN), a bone specific marker, as well as higher ALP activity than control group. In addition, after 4 weeks in vivo implantation, robust bone formation was only observed in ECO group, indicated by higher bone volume (BV) and bone mineral density (BMD),as well as denser staining of alizarin red (means more calcium). In contrast, very limited bone formation was seen in control group.

Matrix-bound Nanovesicles for Treatment of Achilles Tendinopathy

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Despite advancements in surgical techniques, treatment options for damaged or diseased tendons are still plagued by complications such as adhesions, loss of strength, and chronic pain. Tendon pathology is often caused by repetitive overuse and strain, resulting in cumulative microtears in the tendon without sufficient time for complete healing and matrix turnover. Current treatment options for tendinopathy include rest, resistance exercise, nonsteroidal anti-inflammatory drugs (NSAIDS), and platelet rich plasma (PRP) therapy; however, there is no consensus on the preferred therapeutic option due to lack of proven data and insufficient understanding of tendon pathophysiology. The present study proposes a tissue engineered, regenerative medicine approach that utilizes molecular components of mammalian extracellular matrix (ECM) for tendinopathy. Bioscaffolds composed of ECM have been widely used clinically as templates for inductive tissue remodeling in multiple anatomic sites, including rotator cuff, Achilles, tibialis posterior, and patellar tendons. The observed constructive tissue remodeling may be due, in part, to the release of nanometer-sized membranous vesicles called matrix-bound-nanovesicles (MBV). MBV are embedded within the fibrillar ECM network and contain potent biologically active signaling molecules that mediate stem cell differentiation and macrophage activation. The rationale for the present study is based upon the recent discovery of Lysyl Oxidase (LOX) as an MBV surface protein. LOX belongs to a heterogeneous family of extracellular enzymes that catalyze the covalent cross-linking of collagen, resulting in stabilization of collagen fibrils. LOX has the potential to not only regulate gene transcription, but to also modify the ECM by affecting collagen cross-linking. However, the role of MBV-associated LOX in wound healing and in particular, tendinopathy, has yet to be determined.

Preliminary data suggest that cellular uptake of MBV-associated LOX results in increased expression of collagen type III, which is commonly associated with wound healing. It is hypothesized that ECM-scaffold degradation and release of MBV may initiate a feedback loop to coordinate the synthesis and deposition of matrix components. By utilizing the functional role of LOX in cross-linking collagen and regulating matrix deposition via gene expression, MBV may therefore represent an attractive method to enhance the biomechanics of remodeled tissue, especially its tensile properties. Although there has been much interest in utilizing LOX proteins in regenerative medicine applications, these efforts have been confounded by the low solubility of LOX, which often necessitates urea purification buffers and lengthy refolding procedures. MBV provide not only a feasible method for purifying innate extracellular LOX proteins, but also offer a conceptual model for how LOX is incorporated into the matrix. The proposed study will characterize the differential expression of LOX and its various isoforms (LOXL 1-4) utilizing MBV, which themselves will be isolated from ECM bioscaffolds from multiple source tissues. Further, the study will evaluate the functional and regulatory roles MBV-associated LOX plays in repairing damaged tendons.

Organ-on-a-chip System for the Modeling of Synovial Joint Pathologies

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Trauma, inflammation, infection, and aging cause damages to joint tissues, ultimately leading to degenerative disorders, such as osteoarthritis (OA), resulting in painful physical disabilities that compromise quality of life. However, currently there exists no effective disease-modifying medications (DMMs) for OA treatment. Herein we propose the engineering of a stem cell-based 3D human micro-joint chip (mJoint), which is physiologically analogous to the native joint, and capable of modeling pathogenesis of joint diseases for DMM screening/development. Different joint tissues, including osteochondral complex (bone and cartilage), synovium, and fat pad were engineered from human mesenchymal stem cell (hMSC)-laden gelatin hydrogel scaffolds and prepared as modules for convenient integration in the bioreactor. The 3D tissues were matured by differentiating them in their respective induction media for 28 days before their integration in the mJoint bioreactor. The interconnection among tissue components was guided via directional fluidic flow of the culture medium to simulate in vivo physiology. RT-PCR and histological staining both confirmed that individual joint components within the mJoint chip were able to maintain respective tissue-specific phenotypes up to 4 weeks. As an attempt to model OA in the mJoint, inflamed synovium-conditioned medium was perfused through cartilage tissues. This led to markedly reduced expression levels of major cartilage markers and significantly upregulated genes associated with cartilage matrix degradation. The results indicated the generation of degenerative arthritis in mJoint chip. We are including macrophages into the different tissues to better simulate the physiology under different conditions. To the best of our knowledge, this study is the first to report the generation of human cell-derived multi-tissue joint chip that allows the study of joint pathologies. In addition, this novel chip allows the generation of OA models with different etiologies, through applying different insults and targeting different tissues, thus assisting the screening and development of personalized DMMs.

iPSCs-Derived Osteochondral Tissue Chip to Model Joint Physiology and Osteoarthritis Pathology

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Osteoarthritis (OA) is a common joint disease, which causes pain and immobility. To date there are no efficacious treatments able to retard the progression of OA. The lack of disease modifying OA drugs (DMOADs) may be the function of incongruence between in vitro models of OA and the pathogenesis in vivo, and between disease mechanisms in humans and model animals. To overcome these issues, we developed tissue chips in vitro that functionally represented the osteochondral tissue directly affected by OA from human cells. Compared with MSCs, induced pluripotent stem cells (iPSCs) are a better alternative, since they exhibit an almost unlimited proliferative capacity in culture, and stable and consistent pluripotent potential to differentiate into all MSC lineages. In this study, we aimed to generate osteochondral tissue chips from iPSCs and use this effective model to study the OA pathology and develop DMOADs. iPSCs were first differentiated into MSC-like cells (iMPCs), whose stemness were tested to be comparable to the primary mesenchymal stem cells through colony-forming unit assay and tri-lineage differentiation. Then, iMPCs were induced into osteochondral tissues in customized bioreactors with two chambers. The upper chamber was perfused in chondrogenic medium and lower chamber was perfused in osteogenic medium. After 28 days, the top component showed higher expression of chondrogenic markers COL2 and AGG, and strong alcian blue staining, while bottom sides exhibited higher expression of osteogenic markers OCN, OPN, BSPII, and positive staining of alizarin red. These results indicated the cartilage formation in the top part and bone formation in the bottom part of the constructs. The bi-phase osteochondral tissues were successfully generated in this bioreactor system. In the next step, osteoarthritic osteochondral tissue will be made to simulate the human pathological process, which provides a good platform to examine the efficacy of several potential DMOAD under disease-like condition.

Cord Blood-Derived Endothelialization of Tissue-Engineered Vascular Grafts

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Introduction: Coronary heart disease causes 370,000 deaths annually. To overcome the limited availability of autografts, tissue-engineered vascular grafts (TEVGs) have become an alternative. Given the nature of rapid availability and enriched progenitor cells, cord blood has become an attractive cell source. Previously, we found that human cord blood-derived endothelial cells (HCBECs) have a significantly higher proliferation rate compared to other endothelial cells. In this study, we examined the application of HCBECs in developing a functional TEVG.

Methods: HCBECs were isolated using an approved IACUC protocol. Polycaprolactone/gelatin/fibrinogen electrospun scaffolds were fabricated and surface modified with thermoforming and a dual-coating of collagen type IV and fibronectin. Cell characterization was performed using flow cytometry and immunochemistry. Scaffolds were characterized using scanning electron microscopy; porosity and fiber diameter was quantified. Cell proliferation, cell attachment, and platelet adhesion on cell-seeded scaffolds were evaluated in vitro.

Results: HCBECs are positive for endothelial cell markers CD31 and CD105 while negative for hematopoietic marker CD45. Surface modification with thermoforming and coating reduces scaffold porosity, enhances HCBEC attachment, and decreases platelet adhesion. We are currently evaluating the cell-seeded scaffolds under physiological fluid flow in a bioreactor and in immuno-deficient mice.

Development and assessment of a novel tissue engineered mitral valve with an engineered chordal apparatus

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Valvular heart disease is a major source of morbidity and mortality around the world, and mitral valve regurgitation and mitral valve stenosis [1] are diseases which result in valve repair or replacement. Current valve replacement with mechanical or bioprosthetic valves is burdened by chronic anticoagulation therapy or limited durability, respectively [2]. Additionally, these prosthetic valves are unable to remodel and cannot support somatic tissue growth in younger patients, often requiring repeat operations. Tissue engineered heart valves have been proposed to overcome the limitations of anticoagulation and calcification of the prosthetic valves by utilizing biodegradable scaffolds that rely on endogenous tissue growth to augment the engineered valve [2,3]. In this study, a bi-leaflet, fully-assembled, stentless, electrospun tissue engineered mitral valve was developed with an engineered chordal apparatus. Electrospun double component deposition was used as the bioprocessing method to achieve valve anatomical geometry as well as leaflet physiological microstructure and mechanics. Engineered chordae tendineae was fabricated using a novel mandrel-less electrospinning technique and fixed at the valve commissures. Valve mandrel design was optimized using computational modeling of the mitral valve with a chordal apparatus. Chordae tendineae number, branching, length, and anchoring position are being studied to enhance leaflet functionality. Additionally, leaflet and chordae attachment techniques are being evaluated to avoid rupture at the attachment site. A pulse duplicator is being used for in vitro functional assessment of the mitral valve with varying chordal apparatus configurations. Finally, acute large animal studies are being conducted to assess model viability, leaflet motility, thrombus formation, and structural integrity.

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Cytokine Mimicking Microspheres for Use in Porous Scaffolds

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Background: Cardiovascular disease is the number one cause of death in the US and treatment of this disease often requires the use of a vascular graft. Research towards the development of a tissue engineered vascular graft (TEVG) has shown promising results of previous studies utilizing stem cells yet many limitations accompany the use of cells. This study focused on determining if the pro-remodeling properties of TEVGs could be replicated in a biomimetic graft seeded with microspheres that release bioactive factors.

Methods: Bioactive factors known to promote remodeling were loaded into biodegradable microspheres and seeded into poly(ester urethane) urea (PEUU) scaffolds. The seeded scaffolds were release for 21 days with samples of the supernatant collected daily. The release profiles of the microspheres and seeded scaffolds were determined using a bicinchoninic acid assay and ELISA kits (IL-8 and vascular endothelial growth factor) testing the supernatant samples. The toxicity of the supernatant was also tested using a LIVE/DEAD assay. To test for uniform retention in vivo, a scaffold was seeded with microspheres loaded with fluorescein isothiocyanate (FITC) and implanted as an aortic graft in Lewis rats for 3 days after which they were explanted and assessed for remaining microspheres.

Results: A linear release of cargo from the scaffolds over a 10 day period was observed from the BCA and ELISA assays. No cellular death was observed due to the microsphere releasates over the course of 24 hours. Preliminary studies also showed that the microspheres remain within the graft after exposure to physiological flow in vivo after 3 days. FITC-loaded microspheres alone were insufficient to prevent acute clotting, validating the need for pro-remodeling agents within synthetic scaffolds.

Conclusion: These pilot studies suggest that our bioactive factor-loaded microsphere approach to TEVGs could be a viable alternative to current cell-based TEVGs.

Combining a Peripheral Nerve Matrix Derived Hydrogel and Post-Surgical Therapy for Improving Functional Recovery Following Nerve Reconstruction

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In the US, peripheral nerve injury (PNI) affects an estimated 20 million people, totaling nearly $150 billion yearly in health-care costs. Without intervention, peripheral nerves show a slow and lacking regenerative response following injury, making surgical intervention an imperative. Creating a therapy to both increase the rate of regeneration as well as the extent of function regained is of great clinical interest. A novel peripheral nerve-specific extracellular matrix (PNM) hydrogel has been shown to increase constructive remodeling of injured peripheral nerves, and this study primarily aims to observe the PNM hydrogel's efficacy in a rat sciatic transection model. Also, this study aims to observe potential changes in the PNM hydrogel's efficacy by incorporating post-surgical therapy. This rationale is based on electrical stimulation therapies having shown to increase nerve recovery when used in conjunction with standard nerve repair techniques. Data will be collected up to 24 weeks post-surgery using sciatic functional index, end-study electrophysiology metrics, and histological analysis to assess differences in nerve regeneration with varied treatments and age of the animal. Initial data from a rat sciatic crush model shows that the nerve gel increases the rate of recovery, matching positive controls quicker than groups treated without the gel. Up through 12 weeks of the 24 total weeks have been completed by all rats entered into the study. Kinematic metrics have shown recovery up through 10 weeks post-surgery with no significant differences observed between repair groups with and without the PNM hydrogel. While this study focuses on a transection injury model, we are also conducting parallel studies to observe the PNM hydrogel's efficacy in crush and gap models. Furthermore, we are conducting this study to create a multi-faceted therapy to regenerate peripheral nerves more effectively by inclusion of post-surgical therapy.

Simultaneous Heart and Lung Co-differentiation by Modulating WNT, Activin A and BMP4

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Background: The mesoderm-derived heart and endoderm-derived lung are the two major organs within the chest cavity, and their mutual interactions are essential for their proper morphogenesis and functional maturation. Human induced pluripotent stem cell (hiPSC)-based models have dramatically advanced our understanding of human heart and lung morphogenesis. However, these models are limited to studying one parenchymal lineage at a time.

Objective: This study aims to recapitulate the co-developmental of heart and lung during embryogenesis through simultaneous induction of cardio-pulmonary lineages from hiPSCs.

Methods: Co-differentiation of heart and lung was divided into three main stages: Stage 1 (primitive streak), Stage 2 (foregut endoderm, cardiac mesoderm) and Stage 3 (pulmonary and cardiac progenitors). In this study, we focused on optimizing factors affecting Stage 1 after circumspectly reviewing the signalling pathway crucial for both heart and lung organogenesis. The differentiation was terminated on Day 10 and the co-differentiation efficiency was evaluated by immunofluorescent staining with NKX2.1 and NKX2.5 antibodies, which targeting early lung and heart progenitors, respectively.

Results: Initial exposure of iPSCs to CHIR99021 was dose- and time-dependent. We observed that 24-h CHIR99021 treatment at 6 [?]M is sufficient to drive mes-endoderm induction and subsequently cardio-pulmonary specification. Lower or higher CHIR99021 did not improve the outcome, regardless of time of exposure. We found that addition of 100 ng/mL Activin A in Stage 1 promoted higher lung differentiation efficiency, while the addition of BMP4 enhanced cardiac differentiation. By keeping BMP4 at a constant concentration of 10 ng/ml, addition of 10 ng/mL Activin A and above compromised cardiac differentiation but did not further improve lung induction. Our results showed that 5 ng/mL Activin A in combination with 10 ng/mL BMP4 yielded highest efficiency for heart and lung progenitor differentiation. Staining with FOXA2, SOX17, and VEGFR2 confirmed the concomitant induction of definitive endoderm and mesoderm on day 4 after induction.

Conclusion: This preliminary study demonstrates the feasibility of simultaneous co-differentiation of heart and lung progenitors by modulating WNT, activin A and BMP4.

Differentiation of hPSCs into Islet-mimetic cells: Encapsulation versus Suspension Culturing

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Type 1 diabetes treatment currently relies on daily exogenous insulin therapy, but patients still experience long term negative side effects from imperfect blood glucose management. A long-term treatment involves transplantation of insulin-producing pancreatic islet cells; however, lack of available donors limits the use of this technique. An alternative source for implantation can be provided by a scalable differentiation of human pluripotent stem cells (hPSCs) into insulin producing cells. Here we compare the three-dimensional culturing techniques of alginate-encapsulated (AE) hPSCs with stirred-suspension (SS) hPSCs for differentiation of islet-like cells. The goal of this project is to discern the best means of scalable culture of glucose-sensitive insulin secreting cell differentiation. Previous work from our group has shown enhancement of islet specific differentiation under AE culture over adherent culture, while other groups have shown successful SS culture and differentiation (Pagliuca et al., Cell, 2014). Both differentiation protocols provide a scalable culture with the potential to solve the low islet availability of pancreatic donors. However, the AE culture has specific advantages over SS culture, in preventing overaggregation of the cells, protecting the cells from bioreactor hydrodynamics, in addition to providing biophysical cues for differentiation. In this project we are developing a stage specific comparative evaluation of AE differentiation against SS differentiation.

Development of human organotypic culture models for teratogenesis assessment on limb development

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The limb-bud high-density micromass culture using chick, mouse or rat cells has been a useful tool in studying chondrogenesis and early skeletal development, but its predictive value in teratological studies has proven poor. We thus prepared micromasses using human cells, in particular mesenchymal stem cells (hMSCs) transduced with a lenti-viral Collagen II promoter-driven GFP reporter for non-invasive analysis of chondrogenesis. hMSC-based micromasses were harbored in a fluidically-enabled bioreactor and co-cultured with HUVECs encapsulated in a photocrosslinkable hydrogel, generating a hMSC/HUVEC organotypic culture model (OCM) of limb bud chondrogenesis. We also developed a hMSC-based culture to study joint formation in a mechanically activated bioreactor. We challenged the hMSC/HUVEC OCM with two known teratogens: valproic acid and thalidomide, to test the use of this model as a predictor of environmental toxicant teratogenic potential. The hMSC/HUVEC OCM demonstrated robust chondrogenesis, indicated by increased alcian blue staining and Collagen II-Aggrecan immunohistochemistry at stage-specific gene expression over that of hMSC OCMs. Lenti-viral engineered OCMs showed similar trends in terms of fluorescence intensity and gene expression fold change, revealing the feasibility of non-invasive monitoring of the system. Valproic acid, an HDAC inhibitor, significantly reduced hMSC-only limb bud OCM chondrogenesis – consistent with its role in epigenetic reprogramming. Thalidomide did not show any detectable impact on hMSC-only limb bud OCM chondrogenesis. However, thalidomide significantly inhibited HUVEC viability/tubule formation and hMSC chondrogenesis in hMSC/HUVEC limb bud OCMs. This result is consistent with thalidomide-induced inhibition of angiogenesis in vivo and subsequent limb reduction in humans. Joint formation modeling cultures also showed robust chondrogenesis, which was reduced due to mechanical activation, as it happens in vivo, with the parallel expression of joint segmentation markers (i.e. GDF5). We conclude that the novel hMSC-HUVEC limb bud and hMSC joint OCMs represent a reproducible and controlled model to screen for potential limb teratogens.

Novel Three-Dimensional Fuzzy Graphene (3DFG)-Based Ultra Microelectrodes Array for Sub-Cellular Electrical Recordings

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Investigating electrical activity of cardiomyocytes and neurons is essential to understanding their physiology and mechanisms of cardiac and neurological diseases. Microelectrode arrays (MEAs), at the moment, represent the gold standard for extracellular monitoring of electroactive cells since they enable long-term multi-site recordings at sub-msec temporal resolution, provide two-way interaction with the cells, and have well-established microfabrication methods. However, currently used planar MEAs are limited by their inability to record and stimulate at a single cell and sub-cellular resolution since reducing the electrode surface area to single cell dimensions leads to a significant increase in impedance. High impedance affects the recording capabilities of the electrode by reducing the signal-to-noise ratio (SNR) of the recorded signal. Single cell/sub-cellular recording is necessary to (i) enable high density electrode arrays, (ii) provide high precision and spatial resolution, and (iii) prevent averaging of signals from multiple cells.

To overcome this limitation, we developed three-dimensional fuzzy graphene (3DFG)-based microelectrodes. 3DFG involves out-of-plane growth of single-to-few layers of graphene flakes, thus leveraging graphene's outstanding surface-to-volume ratio. The high surface area of 3DFG leads to better cell-electrode interactions, and significant reduction in impedance without the need of any surface coating modifications. 3DFG microelectrodes demonstrated a 20-fold reduction in the impedance compared to planar monolayer graphene of similar geometric area. The low impedance of 3DFG microelectrodes led to electrical recordings of human embryonic stem cells-derived cardiomyocytes with high SNR. Furthermore, the lower impedance values of 81 μm^2 3DFG electrodes compared to 2500 μm^2 planar graphene electrodes demonstrates the feasibility to develop ultra-microelectrode arrays to perform sub-cellular measurements of cardiomyocytes and neurons. Our presented approach would greatly impact our basic understanding of signal transduction in complex cellular assemblies in health and disease. Furthermore, it would provide a platform for developing and screening of therapeutics.

Adipose-Derived Stem Cells Partially Mitigate Muscle Atrophy after Peripheral Nerve Injury in the Rodent Model

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Muscle recovery after long-gap peripheral nerve injury poses an exceptional challenge for regenerative medicine. Nearly $150 billion is spent annually on peripheral nerve injury and its related ailments such as muscular atrophy after denervation. Successful regrowth of the injured nerve does not necessarily lead to regeneration of muscle, especially if combinative therapies addressing both the nerve injury and the muscle atrophy are not used. Here, a rodent model for muscle atrophy of the gastrocnemius results from a 1.5cm defect to the sciatic nerve. To address the nerve injury, an autograft was placed into the defect consistent with the standard of care. Additionally, allogeneic rodent adipose-derived stem cells were injected into the gastrocnemius post-operatively in two cohorts, one receiving a single injection, and the other receiving two injections, post-operatively and at three weeks. At six weeks, the cohorts having received an ASCs injection post-operatively had a higher muscle mass percentage retained, had larger average fiber area, and was shown to have less overall lipid content accumulated throughout the musculature. Additionally, muscles having received ASCs injection showed increased presence of IL-10 (anti-inflammatory cytokine) and Ki67 (cell proliferation marker), with decreased presence of iNOS (pro-inflammatory cytokine). Collectively, this investigation is suggestive that an ASC injection into denervated muscle post-operatively is able to partially mitigate the onset of atrophy as determined by relative muscle mass measurements being corroborated by average fiber area size. Further, IL-10 appear to have greater upregulation in the ASC-injected condition, suggestive of being further in the healing cascade.

Establishing the role of ECM stiffness in skeletal muscle regeneration

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Many studies have shown that tissue becomes stiffer with age due to increased fibrogenesis and extracellular matrix (ECM) deposition. This stiffness is due, at least in part, to crosslinking of collagen in the ECM. These alterations are important, as studies have consistently shown that mechanical properties of the microenvironment are critical drivers of stem cell responses. Given that skeletal muscle regeneration is impaired with aging as a result of aberrant muscle stem cell (MuSC) lineage specification, we hypothesize that regenerative capacity can be improved by altering aged skeletal muscle stiffness to mimic those of young tissue.

First, to understand the effect of age on matrix biophysical properties, we performed an in-vitro study. Young and old mouse myofibroblasts were isolated and allowed to elaborate a matrix for three weeks. After decellularization, the topology and stiffness of the matrix was measured by atomic force microscopy (AFM). Analyses revealed that topology was markedly different between young and old matrix. The old matrix was also found to be ~3 times stiffer than young matrix.

Next, to evaluate whether modulating muscle stiffness in aged mice improves muscle regeneration after injury, we injected old mice daily with beta-aminopropionitrile (BAPN), an inhibitor of the collagen cross-linker, lysyl oxidase (LOX). After four weeks, muscles were injured by cardiotoxin, and allowed to recover for another two weeks. There was a significant improvement in muscle recovery after injury in BAPN-treated mice compared to age-matched saline control mice, as determined by higher specific force production and increased regeneration index. Half relaxation time was also closer to that of young, indicating that the muscle quality was similarly improved.

The above in vitro and in vivo studies support our hypothesis that increased muscle stiffness with aging contributes to an impaired regenerative response. Future studies will seek to identify the underlying mechanisms for an improved regeneration with decreased muscle stiffness in aged animals. The long term goal of these studies is to aid in the development of therapeutics that might improve muscle regeneration in an aged population.

Enhancing Regenerative Potential of in vitro-expanded chondrocytes by selectively removing senescent cells

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Autologous chondrocyte implantation (ACI) is a biomedical treatment that repairs damages in articular cartilage to provide pain relief and mobility recovery while, at the same time, slowing down the progression to osteoarthritis. However, the clinical outcome of ACI is still variable, and the quality of chondrocytes may account for one of the major reasons. In order to collect a sufficient number for transplantation, isolated chondrocytes usually undergo an extensive in vitro expansion (from 0.1 million to more than 40 million). During this culture period, the proliferative capacity and regenerative potential of chondrocytes was declining, known as dedifferentiation. In addition, the number of senescent cells also increased with the culture, which not only lost the repair capacity but also displayed a senescence-associated secretory phenotype (SASP), which together adversely affected the quality and quantity of new cartilage after implantation. Therefore, selectively removing senescent cells would be a potential avenue to improve the quality of chondrocytes and the efficacy of ACI. Recently, FOXO4-DRI, a FOXO4 peptide that perturbs the FOXO4 interaction with p53, was reported to selectively kill the senescent cells through triggering apoptosis [1]. In this study, we performed the chondrocyte isolation and in vitro expansion, which are similar to those observed in ACI. We then characterized the chondrocytes that were cultured for a very short period after isolation, or that have been extensively expanded for the implantation, with special attention to the ratio of senescent cells. We finally examined the effect of FOXO4-DRI on chondrocytes. We hypothesized that the number of senescent cells increased with culture time, as indicated by the higher SASP-associated gene expression and more staining of senescence-associated beta-galactosidase (SA-βgal). We also hypothesized that FOXO-DRI could selectively remove the senescent cells in chondrocytes through apoptosis and enhanced the regenerative potential of chondrocytes.

Islet-mimetic Organoid Vascularization using Microvessel Fragments

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Islets of Langerhans are vital for the maintenance of the body's glucose levels through the functionality of the insulin secreting beta cells. Diabetes forms when insulin production isn't high enough, whether through beta cell destruction (type 1 diabetes) or insulin resistance (type 2 diabetes). The leading long term solution for increasing insulin production is through transplanting primary human islets, which is limited due to donor scarcity. Alternatively, islet mimetic organoids could be used for implantation. Organoids are in vitro synthesized tissue that mimics the structure and functionality of in vivo organ systems. Primary islets are constructed of endocrine cells, stromal cells, and a dense vascular network. The aim of the current project is to form an islet-mimetic organoid that maintains glucagon and insulin secretion with the integration of an intra-islet vascular network. Hence we hypothesize that the resulting organoid will replicate the structure and functionality of primary human islets.

A crucial step for islet organoid engineering is the controlled aggregation into a 3-D spheroid morphology. Our lab has developed methods for engineering controlled 3-D heterotypic (different cell types) aggregation of human pluripotent stem cell (hPSC) -derived pancreatic endocrine cells and endothelial cells. The intra-vascular network was reproduced by aggregating hPSC derived pancreatic endocrine cells, adipose-derived microvascular fragments, and stromal cells. The formation of the neo-vascular network has found to be sensitive to the phenotype of the hPSC-derived cell population and the culture media. The resulting vascularized organoids demonstrated higher gene expression of maturing pancreatic beta cell markers (NKX, PDX1, and Insulin), an islet specific endothelial gene (API), and an endothelial diaphragm fenestration indicator (PLVAP) compared to homotypic aggregates of hPSC-derived pancreatic endocrine cells. With enhanced pancreatic phenotype and vascular network, these organoids will be highly applicable in regenerative therapy for diabetes and incorporated into microphysiology system models for disease modeling.

Perfused 3D Printed Collagen Tubes Support Tissue Viability

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Background: Engineering large volume tissues to repair or replace damaged tissues is often limited by a lack of a vasculature. Currently, many biomaterials are used fabricate vessels but developing 3D branching structures capable of providing nutrients to a large volumetric tissue is challenging. 3D printing has the potential to address these problems; however, printing soft, deformable biomaterials into complex architectures is difficult. Here, we utilize Freeform Reversible Embedding of Suspended Hydrogels (FRESH), an embedded 3D printing technique, to print type I collagen, and as proof of concept, we 3D print a vascular tube and determine its effectiveness at supporting tissue viability.

Methods: Collagen tubes (ID: 1.4 mm, Wall thickness: 300 um) were 3D printed using FRESH. Fluorescent dextrans (MW 10,000 and 70,000) were perfused through the tube to confirm patency and permeability. To verify that media perfusion through the tube could support tissue viability, diffusion-limited tissues consisting of 37.5 million C2C12s/mL in a collagen I/Matrigel mixture were cast around the tube and either maintained in static culture or perfused (0.4 mL/min). Bulk cast tissues, without a collagen tube, were maintained in static culture and served as a control for tissue viability studies. At day 5, tissue cross-sections were stained with LIVE/DEAD to assess viability.

Results: Collagen tubes were patent and showed increased diffusion of the low MW compared to the high MW dextran. Static culture and bulk cast tissues displayed a non-viable interior with a thin layer of viable cells near the tissue surface. In contrast, the interior of perfused tissues had predominantly live cells suggesting that perfusion through the tube can support tissue viability.

Conclusions: Collagen tubes can be 3D printed and perfused to support tissue viability. Future work will include printing and perfusing more complicated vessel networks and use of cells with a higher metabolic demand, like cardiomyocytes.

Metabolic Labeling and Chemoselective Functionalization of Native Biomaterials

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Attributed to the preserved extracellular matrix (ECM) compositions, decellularized biomaterials are commonly used for tissue and organ bioengineering and for reconstructive surgical applications. Immobilization of bioactive molecules onto ECM biomaterials can modulate the ECM-cell interaction and facilitate tissue regeneration. Here, we report a metabolic glycan labeling technology that covalently incorporates azide ligands into the ECM of native tissues and organs both in vivo and ex vivo using Ac4GalNAz. The incorporated azide ligands remained stable after decellularization and enabled ECM functionalization with alkyne-modified biomolecules via the click reaction. Using alkyne-modified heparin as a model, we demonstrated that biomolecules retained their bioactivity after click immobilization onto azide-labeled ECM. As the next step, we are working on immobilizing complementary single-stranded DNA oligos onto decellularized ECM and cell surface, with the goal of facilitating efficient and homogeneous cell-to-ECM engraftment via DNA hybridization.

Transdermal Delivery of Extracellular Vesicles Using Dissolvable Microneedle Arrays to Control Inflammation

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Curcumin-loaded extracellular vesicles (EVs) have shown promising outcomes in controlling inflammation in preclinical models. However, systemic delivery of therapeutic EVs is compromised by their short plasma half-lives with the consequent need for multiple daily injections that could potentially reduce patient compliance and increase treatment cost. Transdermal delivery of EVs offers advantages for the patient, including its minimally invasive, pain free delivery as well as convenience of delivery, and the avoidance of first pass metabolism and gastrointestinal degradation. Here, for the first-time, dissolvable microneedle arrays (MNAs) were investigated as a system for localized delivery of curcumin-loaded EVs. EVs were isolated from murine macrophage cell line (J774A.1) using size exclusion chromatography and characterized using dynamic light scattering, tunable resistive pulse sensing, transmission electron microscopy and western blotting. As a novel strategy, prior to curcumin loading, EVs were loaded by sonication with albumin to improve the retention of encapsulated curcumin. Curcumin binds to the hydrophobic pocket in albumin and improves its retention within the EV lumen. MNAs were fabricated from carboxymethylcellulose (CMC) via micromilling/spin-casting method in the form of obelisk shape microneedles with curcumin-loaded EVs encapsulated in the needle tips. EVs released from MNAs were readily taken up by HEK293 and NIH3T3 in a time-dependent manner and maintained their biological activity of downregulating NF-kB activation in a reporter RAW 264.7 cell line. Furthermore, MNAs efficiently delivered curcumin-loaded EVs to the dermis of rat skin with clinically applicable release profiles. To evaluate MNA delivered EV function in vivo, we co-delivered bacterial lipopolysaccharide and curcumin-loaded EVs and evaluated inflammation-related protein expression profiles in the rat skin over time. MNAs with curcumin-loaded EVs reduced key biomarkers of inflammation including TNF-α, IL-1β, IL-6, IFN-gamma, ICAM-1, L-selectin, MCP-1 and TIMP-1 expression. Taken together, these results demonstrate efficient and biologically effective MNA delivery of therapeutic EVs to the intradermal microenvironment of rodent skin and support the development of MNA mediated exosome delivery for clinical applications.

Novel Delivery System of TGF B-1 utilizing fabricated scaffold for Bone Regeneration of Compromised Wounds in a Swine Model (Sus scrofa)

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Background: Modern battlefield injuries frequently result in complex composite tissue defects. Open compromised wounds with significant tissue loss are sub-optimal for bone regeneration. Currently, there is no ideal therapy available to regenerate large bone volumes in compromised wounds. Our technology, which aids in the acceleration of bone healing, is a biologic device which controls inflammation and stem cell differentiation at the site of injury. Our goal was to demonstrate translational potential using a swine model of a novel delivery device technology designed to regenerate bone for compromised extremity wounds.

Methods: Adult female Yucatan swine were utilized for evaluating in vivo drug delivery technology using a 3 cm critical bone defect model. End-point was designated at 30 days. X rays of hind limbs were performed every 2 weeks. Blood work, flow cytometry, and animal assessments were performed on designated post-operative days. At the study endpoint, regenerate tissue was collected and evaluated.

Results: Animals experienced no systemic effects from treatment. Histologic evaluation of regenerate tissue demonstrated TGFβ-1 scaffold treatment caused significant woven bone formation. Untreated defects demonstrated woven bone near osteotomy site, but no bone in the defect proper. Woven bone contained high osteocyte density and active osteoid surfaces lined with osteoblasts. INFUSE also yielded regeneration, but with more cartilage, and also yielded ectopic bone. Immunohistochemistry of the regenerate tissue showed no M1 inflammatory macrophages in Gelatin Sponge +TGFβ-1 treated defects. INFUSE treated defects showed M1 cell infiltration.

Conclusion: Military personnel are substantially burdened with traumatic bone injury to the extremities, but no ideal therapy is available to regenerate large bone volumes. Controlled delivery of TGF β -1 promotes bone regeneration in critically sized bone defects. We believe targeting the immune/inflammatory response in the injury site is a critical point in acute care where potential for healing may be improved; improving outcomes of patients with these injuries.