2018 McGowan Retreat
Poster Abstracts

Cellular and Gene Therapy

1. **Matthew B. Amdahl**, Elin Petersen, Paola Corti, Courtney Sparacino-Watkins, Angela Fago, Mark T. Gladwin and Jesus Tejero. *Interactions of zebrafish cytoglobins with oxygen and nitric oxide*
2. **Colin Beckwitt**, Amanda Clark, Katsuhiko Warita, Zoltan N. Oltvai and Alan Wells. Adjuvant Statin Therapy *Efficacy is dictated by Tumor Dormancy and Statin Lipophilicity in ex vivo and in vivo Models of Metastatic Breast Cancer*
3. **Michael R. Behrens** and Warren C. Ruder. *Engineering 2nd-Messenger Control Mechanisms for Cellular Programming*
4. **Debra Bourne**, Francesco Egro, Jacqueline Bliley, Isaac James, E. Michael Meyer, Vera Donnenberg, Albert D. Donnenberg, Barton Branstetter, Kacey Marra, Sydney Coleman and J. Peter Rubin. *Stem Cell Therapy Enriched Fat Grafting for the Reconstruction of Craniofacial Deficits*
5. Rachael Kreft, Laura Nistico, Barbara Byers, Nick Giannoukakis, Christopher Post, **Francis Cartieri**, Tracy Spirk and Howard Edington. *Biofilm associated infections, inflammatory response, and melanoma progression*
6. **Patrick G. Chan**, Julie A. Phillippi, Marie Billaud and Thomas G. Gleason. *Role of Calpain in Bicuspid Aortic Valve Aneurysmal Disease*
7. **Evan Delgado**, Shu-Hao Hsu, Junyan Tao, Satdarshan Monga and Andrew Duncan. *Regulation of Oncogenic Signaling Pathways in Hepatocellular Carcinoma by the Pleiotropic Scaffold Protein IQGAP1*
8. **David Gau**, Taber Lewis, Lee McDermott, Peter Wipf, David Koes and Partha Roy. *Structure-based virtual screening identifies small molecule inhibitor of the profilin1-actin interaction with anti-angiogenic properties*
9. **Chuanyu Guo**, Jian Zou, Yi Wen, Wei Fang, Donna Beer Stolz, Ming Sun, Xiangyun Wei. *Apical cell-cell adhesions reconcile symmetry and asymmetry in zebrafish neurulation*
10. **Lauren V. Huckaby**, Marie Billaud, Jennifer C. Hill, Tara D. Richards, Julie A. Phillippi and Thomas G. Gleason. *Estrogen Receptor Expression in the Non-aneurysmal and Aneurysmal Human Thoracic Aorta*
11. **Johnson, Zariel I.,** Willis, Monte S., Jaynes, Jesse and Yates, Cecelia C. FIBROKINE(TM) Peptides: A Broad-Spectrum of Anti-Fibrotic Chemokine Peptides to Treat Organ Fibrosis
12. **Zariel I. Johnson**, Samuel LoPresti, Brandon Lantonio, Alan Wells, Nahed Ismail, Bryan Brown and Cecelia C. Yates. *Bi-directional Macrophage-Fibroblast Crosstalk Directs Wound Resolution Factors*
13. **Karis Kosar** and Kari Nejak-Bowen. *Treatment of a Mouse Model of Cholestasis with a Thyromimetic Improves Biliary Injury But Exacerbates Hepatocyte Injury*
14. **Ajay Kumar** and Yiqin Du. *Regenerative Effects of Secretome Derived from Long-term Cryopreserved Stem Cells for Glaucoma Treatment*
15. **Brian Martin**, Beth Gabris, Rajiv Reddy, Amr Barakat, Guillermo Romero, Ansuman Chattopadhyay and Guy Salama. *Cardioprotective Actions of Relaxin*
16. Harman Ghuman, Franziska Nitzsche, Madeline Gerwig, Jeffrey Moorhead, Lauren Grice, Nikhita Perry, Alex Poplawsky, Brandon Wahlberg, Fabrisia Ambrosio and **Michel Modo**. *Sub-additive effects of cell and physical therapy in a rodent model of stroke*
17. **Laura Molina**, Minakshi Poddar and Satdarshan P. S. Monga. *Exploring the Role of YAP1 in Liver Regeneration*
18. **Muhammad U. Nisar**, William E. Klunk and Alberto L. Vazquez. *Changes in neuronal activity are not correlated with vascular reactivity in APP-PS1 mice*
19. **Vishal Rakshe** and Shilpa Sant. *Shape Dependent Effects of Nanomaterials on Macrophage Polarization*
20. **Matthew D. Rannals**, Stephanie Cerceo-Page, Thomas M. Hyde, Joel E. Kleinman, Andrew E. Jaffe, Daniel R. Weinberger, Nathan N. Urban and Brady J. Maher. *Targeted translatome profiling using in utero gene transfer identifies ion channel pathophysiology in a brain development model of transcription factor 4 (TCF4) function*
21. **Chris Reyes**, Li Mo, Danielle Guimaraes, Kelly Quesnelle, Yinna Wang and Sruti Shiva. *Nitrite Regulates Mitochondrial Dynamics to Inhibit Vascular Smooth Muscle Cell Proliferation*
22. **Jacquelyn Russell**, Wei-Yu Lu, Marc Abrams, Kari Nejak-Bowen, Minakshi Poddar, Sucha Singh, Stuart Forbes and Satdarshan Monga. *Lack of Beta-catenin in Hepatocytes Impairs Proliferation and Promotes Liver Progenitor Cell-Mediated Repair in Response to the Choline-Deficient Ethionine-Supplemented Diet*
23. **Elizabeth C. Stahl**, Samuel T. LoPresti, Evan R. Delgado, Frances Alencastro, Patrick Wilkinson, Andrew W. Duncan and Bryan N. Brown. *Age-Related Changes in Metabolism and Inflammation of the Liver*
24. **Kyle Sylakowski**, Austin Nuschke and Alan Wells. *Improving mesenchymal stem cell therapeutic function through interaction with extracellular matrix protein Tenascin-C*

25. **Aaron X. Sun**, Ricardo Londono, Raghunandan A. Avula, Uma R. Chandran, Megan L. Hudnall, Rocky S. Tuan and Thomas P. Lozito. *Differences in Neural Stem Cell Identity and Differentiation Capacity Modulate Divergent Regenerative Outcomes in Lizards and Salamanders*

26. **Anil Tiwari**, Sudha Swamynathan, Nicholas Alexander, John Gnalian and Shivalingappa K. Swamynathan. *Krüppel-like factor 4 (KLF4) Regulates Corneal Epithelial Stratification by Controlling Cell Polarity and Plane of Cell Division*

27. **Wenzhong Wei**, Jing Wang, Dong Wang, Nam Vo and Bing Wang. *Intervertebral disc Degeneration in Duchenne Muscular Dystrophy*

28. **Patrick D. Wilkinson**, Evan R. Delgado, Frances Alencastro, P. Anthony Otero, Maddie P. Leek, Matthew P. Weirich, Nairita Roy, Michael Oertel and Andrew W. Duncan. *A role for polyploid hepatocytes in liver repopulation and adaptation to chronic injury*

29. **Yingfei Xue**, Vinayak Sant, Julie Phillippi and Shilpa Sant. *Patient-Derived Valvular Interstitial Cells Model Reveals the Therapeutic Effect of Shape-Specific Nanoceria against Oxidative Stress Induced-Calcification*

30. **Luke A. Ziegler**, Katrina B. Zougari, Jonathan H. Waters and Marina V. Kameneva. *Analysis of Shear-Induced Erythrocyte Deformation Following Intracellular Content Replacement*

31. **Daniel A. Zuppo**, Maria A. Missinato and Michael Tsang. *Elucidating the roles of cenpf and foxm1 in zebrafish cardiac regeneration*

Computation and Modeling

32. **Reza Behkam**, Julie Barkmeier-Kraemer and Jonathan Vande Geest. *The aorta and vocal fold paralysis: is there a connection?*

33. **Eric Lambert**, Vijay S. Gorantla and Jelena M. Janjic. *Generation of mixture models to predict colloidal properties of triphasic nanoemulsions designed for neuroregeneration support in peripheral nerve injury*

34. **Rami A. Namas**, Maxim Mikheev, Jinling Yin, Derek Barclay, Bahiyyah Jefferson, Qi Mi, Timothy R. Billiar, Ruben Zamora, Jorg Gerlach and Yoram Vodovotz. *An Adaptive, Negative Feedback Circuit in a Biohybrid Device Reprograms Dynamic Inflammation Networks In Vivo*

35. **Ehab Tamimi**, Scott Harrison and Jonathan P. Vande Geest. *Computationally optimizing the compliance of biopolymer tissue engineered vascular grafts*

36. **Lukas Schimunek**, Rami A. Namas, Jinling Yin, Derek Barclay, Fayten el-Dehaibi, Andrew Abboud, Maria Cohen, Ruben Zamora, Timothy R. Billiar and Yoram Vodovotz. *A Single Nucleotide Polymorphism in MPPED2 is Associated with Initial Hypo-Inflammation and Adverse Clinical Outcomes after Trauma*

Medical Devices

37. **Sara Abdollahi**, Eric Markvicka, Carmel Majidi and Adam W. Feinberg. *Wearable pulse oximetry with soft 3D printed sensor and electronics for detecting perfusion anomalies through continuous monitoring*

38. **Timothy N. Bachman**, Ling Tian, Mehdi Nouraie, Courtney Q. Vu, Yousif Shwetar, Lauren Williams, Christopher Link, Michael Boisen, Luigi Lagazzi, Christopher Sciortino, Robert L. Kormos and Marc A. Simon. *Hemodynamic Changes during LVAD implantation may be associated with Subsequent Right Ventricular Failure: Opening the Black Box of the Operating Room*

39. **Moataz Abdulhafez**, Karim Kadry, Mohamed Zaazoue, Liliana Goumnerova and Mostafa Bedewy. *Biomechanical Analysis Of Complications In Head Immobilization Devices For Pediatric Neurosurgery*

40. **Ali Behrangzade**, Ryan A. Orizondo, John A. Kellum and William J. Federspiel. *Development of a Combined Artificial Lung and Kidney*

41. **Ashley Blystone** and Ellen Gawalt. *Triple Functionalization of Titanium Aluminum Vanadium*

42. **Gary Yu**, Filip Istanvic, Xucai Chen, Sruti Shiva, Mark Gladwin, Adam Straub and John Pacella. *Co-administration of Sodium Nitrite during Ultrasound Targeted Microbubble Cavitation Therapy Enhances Nitric Oxide Generation and Increases Microvascular Perfusion*

43. **Dan Crompton**, Shushma Gudla, Prithu Sundd and Marina Kameneva. *Potential rheological treatment of Sickle Cell Disease (SCD) by reducing vaso-occlusion in small vessels*

44. **Garrett Coyan**, Yasumoto Matsumura, Drake Pederson, Samuel Luketich, Vesselin Shanov, Vinay Badhwar, Antonio D'Amore and William Wagner. *Acute In Vivo Functional Assessment of a Novel Bioinspired Scaffold-Based Engineered Heart Valve with Biodegradable Magnesium Alloy Stent*

45. **Eoghan M. Cunnane**, Niall F. Davis, Alan J. Ryan, Jochen Hess, David A. Vorp, Fergal J. O'Brien and Michael T. Walsh. *Developing urinary catheter safety devices and improving tissue engineered urethral scaffolds through an enhanced understanding of human urethral biomechanics*

46. **Firuz Feturi**, Hua Wang, Yevgeny Brudno, Vasil Erbas, Liwei Dong, Huseyin Sahin, Wensheng Zhang, Mario Solari, David Mooney, Raman Venkatramanan and Vijay Gorantla. *A single localized dose of ultrasound-responsive release hydrogel improves long-term survival of a vascularized composite allograft*

47. **Firuz Feturi**, Nitin Joshi, Zhaoxiang Zhang, Vasil Erbas, Liwei Dong, Huseyin Sahin, Wensheng Zhang, Mubin Aral, Praveen Vermula, Mario Solari, Jeffrey Karp, Raman Venkatramanan and Vijay Gorantla. *Enzyme Responsive-Release Hydrogel for Targeted Immunosuppression in Vascularized Composite Allotransplantation*

48. **Firuz Feturi**, Jignesh Unadkat, Yong Wang, Bing Li, Wensheng Zhang, Vasil Erbas, Lewei Dong, Zhaoxiang Zhang, Husyein Sahin, Kia Washington, Mario Solari, Raman Venkatramanan, Alexander Spiess. *Allograft Targeted Delivery of Tacrolimus prolongs Vascularized Composite Allograft Survival with Negligible Blood Levels*

49. **Catherine Go**, Partha Thirumala, Jenna Kuhn, Yanfei Chen, Youngjae Chun and Bryan W. Tillman. *Retrograde Hemorrhage and Ischemic Injury after REBOA in a Porcine Model of Uncontrolled Aortic Injury*

50. **Jooli Han** and Dennis R. Trumble. *Muscle-powered Counterpulsation Ventricular Assist Device (mVAD) for Long-term Cardiac Support*

51. **Aimon Iftikhar**, Alexis Nolfi, Pamela Moalli and Bryan Brown. *A Clinically Relevant Rabbit Surgical Model of Pelvic Reconstruction to Evaluate the Regenerative Immune Response to Novel Surgical Materials*

52. **Riazul Islam**, Steven M. Wellman and Takashi D. Y. Kozai. *In vivo imaging of reactive astrocyte following chronic implantation of microelectrode array*

53. **Jenna Kuhn**, Catherine Go, Youngjae Chun, Yanfei Chen, Sung Kwon Cho, William C. Clark and Bryan W. Tillman. *Damage Control of Caval Injuries in a Porcine Model using a Retrievable RESCUE Stent*

54. **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*

55. **Mark H. Murdock**, Jordan Chang, George Hussey, Nduka Amankulor, Johnathan Engh and Stephen F. Badylak. *Non-neoplastic extracellular matrix components abrogate primary human glioma cell malignancy*

56. **Ryan Orizondo**, Alexandra May, Brian Frankowski, Greg Burgreen and William Federspiel. *In Vitro Characterization of a Modular Pump Lung Capable of Multiple Respiratory Assist Applications*

57. **Ryan Orizondo**, Shalv Madhani, Alexandra May, Brian Frankowski, Sang-Ho Ye, Ergin Kocyildirim, William Wagner, Robert Kormos, Jonathan D'Cunha and William Federspiel. *Successful 30-day Sheep Studies of a Wearable Pumping Artificial Lung*

58. **Evan Richards**, William Federspiel, Xinyu Liu, Sang-Ho Ye, Anjal Sharma and Ritwik Ghosh. *Advancing a System for Generating Universal Blood Plasma*

59. **Akhil Patel**, Samer Zaky, Hongshuai Li, Elia Beniash, Charles Sfeir, Sachin Velankar, Yadong Wang, Donna B Stolz and Shilpa Sant. *RegenMatrix - A Growth Factor-free Bone Graft for Craniofacial Bone Regeneration*

60. **Constance M. Robbins**, Guruprasad Raghavan, Jason Yang, James F. Antaki and Jana M. Kainerstorfer. *Diffuse optical imaging for breast cancer monitoring*

61. **Steven M. Wellman** and Takashi D.Y. Kozai. *Two-Photon Imaging Reveals Processes Extension and Cell Body Migration of Reactive NG2 Glia During Brain Injury*

62. **Liam Wisehart** and Christopher Bettinger. *Evaluation of Device-based Mechanical Interlocking System for Sustained Oral Delivery to the Gut*

63. **Timothy K. Chung**, Yogev Baruch, Kory J. Blose, Aneesh Ramaswamy, Justin Weinbaum, John A. Curci and David A. Vorp. *Magnetic field assisted localization of ADMSCs with iron nanoparticles*

Tissue Engineering

64. **Rosalyn Abbott**. *Adipose tissue engineered model for studying metabolic diseases*

65. **Meer Ahmed**, Kristi Rothermund, Tia Calabrese and Syed Picard. *Scaffold-free Dental Pulp Cell Sheets to Enhance Peripheral Nerve Regeneration*

66. **Catalina Ardila**, Ehab Tamimi, William Wagner and Jonathan Vande Geest. *Tailoring TGFB2 release from electrospun scaffolds for vascular tissue engineering*

67. **Travis Armiger**, Marsha Lampi, Cynthia Reinhart-King and Kris Dahl. *Using Sub-Nuclear Sensors to Determine Mechanical Features of Epithelial Monolayers*

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

69. **Avik Basu**, Kristi Rothermund, Kaitlyn Frey and Fatima N. Syed-Picard. *Scaffold-free Tissue Engineering for Periodontal Regeneration*

70. **Andrew Bradshaw**, Jelena Grahovac, Amanda Clark, Linda Griffith and Alan Wells. *A Systems Model of Human Metastatic Melanoma from Invasion to Colonization Identifies Tenascin-C as a Driver of Resistance and Emergence*

71. **Liza Bruk**, Cuneyt Alper and Morgan V. Fedorchak. *Development of a Novel Topical Controlled Release System for Otic Drug Delivery*

72. **Michael J. Buckenmeyer**, Aimon Iftikhar, Alexis L. Nolfi, Meena Sukhwani, Arvind Venkatraman, Ziyu Xian Kyle E. Orwig and Bryan N. Brown. Bioactive *Ovarian Hydrogels Provide a Novel Biomaterial for In Vitro Follicle Maturation*

73. **Jingming Chen**, Amalie E. Donius and Juan M. Taboas. *Chondrocyte Hypertrophy and Mineralization is Controlled by Hydrogel Composition*

74. **Wendy Chen**, Jila Noori, Lin He, Chiaki Komatsu, Maxine R. Miller, Ian A. Rosner, Wenshang Zhang, Kira L. Lathrop, Joshua M. Barnett, Yong Wong, Bing Li, Mario G. Solari, Charleen T. Chu and Kia M. Washington. *Whole Eye Transplantation: Allograft Survival With Tacrolimus Immunosupression And Comparison To Syngeneic Transplantation*

75. **Amanda M Clark**, Kelly Chan, Collin D Eddington, Manu Kumar, Nikolina Dioufa, Sarah E Wheeler, Raman Venkataramanan, Donna B Stolz, Dave Trumper, Linda G Griffith, Douglas Lauffenburger and Alan Wells. *A multi-organ microphysiological system that models dormant-emergent metastatic breast cancer progression*

76. **Adam R. Chin**, Jin Gao, Yadong Wang, Juan M. Taboas and Alejandro J. Almarza. *Regenerative Potential of Various Soft Polymeric Scaffolds in the TMJ Condyle*

77. **Madeline Cramer**, Jenna Dziki, George Hussey, Heth Turnquist and Stephen F. Badylak. *Role of ECM-associated IL-33 in Functional Cardiac Repair*

78. **Gabriella DiBernardo**, Jacqueline Bliley, Deok-Yeol Kim, M. Asher Schusterman, Matthias Waldner, Ryan Schroth, Christopher Mahoney, Damian Grybowski, Debra A Bourne, Isaac James, Neil Fadia, Anandhini Narayanan, Adam Cottrill, Insiyah Campwala, Victoria McGovern, Kia Washington, Alexander Spiess, Donald Crammond and Kacey Marra. *Biodegradable Conduits for Long Gap Peripheral Nerve Repair in a Non-Human Primate Model*

79. **Francesco M. Egro**, M. Asher Schusterman II, Deokyeol Kim, Damian Grybowski, Isaac James, Latha Satish, Phillip Gallo, Kacey Marra and J. Peter Rubin. *Porcine Infected Partial-Thickness Burn Wound Model*

80. **Leonid Emerel**, George R. Fercana, Marie Billaud, Jennifer C. Hill, Tara D. Richards, Xinzhu Gu, David A. Vorp, William R. Wagner, Julie A. Phillippi and Thomas G. Gleason. *A Novel Bioreactor Model for the Study of Human Aortic Disease*

81. **Harman Ghuman**, Carrinton Mauney, Julia Donnelly, Andre Massensini, Stephen Badylak and Michel Modo. *ECM hydrogel injection for the treatment of stroke: Time course comparison of hydrogel retention and phenotypic characterization of invading cells*

82. **Martin Haschak**, Siddhartha Dash and Bryan N Brown. *Cardiac Extracellular Matrix Aging Impacts Macrophage Phenotype and Function*

83. **Darren G Haskett**, Siddarth Madala, Eoghan M Cunnane, Katherine L Lorentz, Samuel K Luketich, Justin S Weinbaum, Antonio D'Amore, Lauren E Kokai, Kacey G Marra, William R Wagner, J Peter Rubin and David A Vorp. *Development of a Seeding Device for Bulk-Seeding of Cells into a Long "Human-Sized" Scaffold for Tissue Engineered Vascular Grafting*

84. **Jorge Jimenez**, Michael A. Washington, Ken K. Nischal and Morgan V. Fedorchak. *In Vitro Optimization of a Controlled Release Cysteamine Eye Drop*

85. **Anna Kalmykov**, Changjin Huang, Arif Abdullah, Elnatan Mataev, K. Jimmy Hsia and Tzahi Cohen-Karni. *Three-Dimensional Nanodevice Arrays for Exploring Electrophysiology in Engineered Excitable Microscale Tissues*

86. **Yoojin Lee**, Urszula Zdanowicz, George Hussey and Stephen F. Badylak. *Matrix-bound Nanovesicles as Regulators of Achilles Tendinopathy during Healing*

87. **Zhong Li**, Gwendoline Goh, Gary Yam, Donald Tan, Jodhbir Mehta, Khiam Aik Khor, Hang Lin and Rocky Tuan. *Spark Plasma Sintered Titania for Potential Application as a Keratoprosthesis Skirt*

88. **Wen Liu**, Ngoc Pham, Amy Spigelmyer, Nick Giannoukakis, Yong Fan, Ellen S. Gawalt and Wilson S. Meng. *Controlling Release of Antibodies from Skin Transplant using Bioaffinity Coassemblies*

89. **Samuel LoPresti**, Clint Skillen and Bryan Brown. *Radical Inflammatory Species-Decellularized ECM Promotes Antioxidant, Pro-Healing Host Response in Muscle Injury*

90. **Katherine Lorentz**, Darren Haskett, Justin Weinbaum, Morgan Fedorchak, Liza Bruk, Abhinav Acharya, Antonio D'Amore, William R. Wagner, Steven Little and David Vorp. *Determining and mimicking the role of mesenchymal stem cells within an implanted elastomeric vascular scaffold*

91. **Samuel K. Luketich**, Chiara Dal Zovo, Andrea De Nicola, Richard Hoff, Dan McKeel, Salim Olia, Xinzhu Gu, Marina V. Kameneva, William R. Wagner and Antonio D'Amore. *Degradation of engineered polyurethane heart valves in a mechanically demanding environment with variable polyester or polycarbonate soft segments*

92. **Christopher Mahoney**, Alex Gerber, Cayla Imbarlina, J. Peter Rubin and Kacey Marra. Dual Method Verification of Adipogenesis in Cultures Containing an Adipose Derived Delivery System

93. **Tyler Meder**, Bryan Brown, Travis Prest, Clint Skillen and Chloe Kaunitz. *Combining a Nerve Specific Hydrogel and Post-Surgical Therapy for Improving Functional Recovery Following Nerve Reconstruction*

94. **Maxine R. Miller**, Jila Noori, Touka Banaee, Yong Wang, Chiaki Komatsu, Bing Li, Wendy Chen, Kira Lathrop, Joshua Barnett, Joel S. Schuman, Wensheng Zhang, Mario G. Solari, José-Alain Sahel, Andrew W. Eller and Kia M. Washington. *Fluorescein Angiography Used to Study Retinochoroidal Blood Flow in Whole Eye Transplantation*

95. **Alexis L Nolfi**, Daniel Hachim, Aimon Iftikhar and Bryan N Brown. *Fluorescent Tagging of Interleukin-4 for Visualizing In-Vivo Release from Coated Polypropylene Mesh*

96. **Ngoc Pham**, Wen Liu, Amy Spigelmyer, Ellen S. Gawalt, Yong Fan and Wilson S. Meng. *Multiplexing antibodies in vivo using Z15_EAK, an Fc-binding gelation module*

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

98. **Travis A. Prest**, Clint Skillen, Christine Heisler, Ruben Hartogs, Kathryn LaBelle and Bryan N. Brown. *Nerve-Specific Extracellular Matrix Hydrogel Enhances Recovery of a Peripheral Nerve Crush Injury*

99. **Aneesh Ramaswamy**, Rachel Sides, Kaori Sugiyama, Hiromi Yanagisawa, Victor Morell, David Vorp and Justin Weinbaum. *Improved Adult and Pediatric Aortic Elastogenesis Driven by Adipose-Derived Mesenchymal Stem Cell Secreted Factors*

100. **Sahil Rastogi**, Jacqueline Bliley, Guruprasad Raghavan, Daniel Shiwarski, Adam Feinberg, Ge Yang and Tzahi Cohen-Karni. *Graphene-based Biocompatible and Transparent Micro-Electrode Arrays for Simultaneous Electrical and Optical Measurements of Electrogenic Cells*

101. **Lindsey T. Saldin**, Shil Patel, Li Zhang, Luai Huleihel, George S. Hussey, David G. Nascari, Lina M. Quijano, Xue Li, Divya Raghu, Anant K. Bajwa, Nicholas G. Smith, Christopher C. Chung, Ashten N. Omstead, Juliann E. Kosovec, Blair A. Jobe, Neill J. Turner, Ali H. Zaidi and Stephen F. Badylak. *Extracellular Matrix Hydrogel Downregulates Neoplastic Esophageal Cell Phenotype*

102. **BK Schilling**, JJ Yoo, SJ Lee, L Tozzi, D Kaplan, KG Marra and JP Rubin. *Soft Tissue and Drug Delivery System Development for Craniofacial Reconstruction after Injury*

103. **K. Verdelis**, S. Ye, X. Gu, S. Luketich, A. D'Amore, P. Gupta, E.M. Cunnanec, J.S. Weinbaum, B.B. Mandal, F.J. O'Brien, W.R. Wagner and D.A. Vorp. *Optimization of Imaging Conditions and Analysis Methodology for Characterization of Properties and Quality Control of Biomimetic Poly(ester urethane) Urea Scaffolds, Silk Scaffolds and Collagen-Elastin Scaffolds for Vascular and Urethral Tissue Engineering*

104. Luis Hortells, Camille Boufford, Evelyn Garchar, Genevieve Doyon, Thomas Gleason, Dennis Bruemmer and **Cynthia St. Hilaire**. *A Role for Telomerase in Valvular Calcification*

105. **Kevin Woeppel**, Sally Zheng and Tracy Cui. *Nanoparticle Crosslinkers: Increasing the Efficacy of Surface Modification for Neural Implants*

106. **Saigopalakrishna S**. Yerneni, Jennifer C. Hill, George R. Fercana, Stephen F. Badylak, Thomas G. Gleason, Lee E. Weiss, Julie A. Phillippi and Phil G. Campbell. *Biomaterials to Simultaneously Promote Angiogenesis and Lymphogenesis*

Interactions of zebrafish cytoglobins with oxygen and nitric oxide

Matthew B. Amdahl (1,2), Elin Petersen (3), Paola Corti (1), Courtney Sparacino-Watkins (1,4), Angela Fago (3), Mark T. Gladwin (1,2,4) and Jesus Tejero (1,2,4)

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Cytoglobin (Cygb) is a ubiquitously expressed heme protein that has been identified in the genome of most higher vertebrate clades including fish, birds and mammals. Since its discovery, extensive study has revealed many structural and functional characteristics of the human Cygb protein, although its physiologic functions remain unknown. Fish have two Cygb genes (Cygb1 and Cygb2); these proteins show small but significant differences, with Cygb2 being more similar to mammalian Cygbs. To gain insights about Cygb function, we compared the properties of zebrafish cytoglobins to the mammalian proteins.

Human Cygb exhibits high-affinity, cooperative oxygen binding (P50 of roughly 1 torr, Hill coefficient up to 1.6), as well as nitric oxide (NO) scavenging through NO dioxygenation and NO production via nitrite reduction. These reactions may modulate NO signaling in vivo. Reduced cytochrome b5 (CYB5) has been shown to rapidly reduce Cygb. We have reported that human CYB5 efficiently reduces both human and zebrafish Cygbs, suggesting a conserved role for the CYB5 reduction pathway.

In this work, we characterize the two zebrafish Cygbs, measuring key parameters and comparing these results to human Cygb. Specifically, we examine oxygen binding for affinity and cooperativity, NO dioxygenation activity, and the ability of zebrafish CYB5 and CYB5R to reduce zebrafish Cygbs during NO dioxygenation. Oxygen affinity measurements suggest high oxygen affinity, with P50 values of <1 torr for Cygb1 and roughly 4 torr for Cygb2. Cygb2 exhibits high cooperativity, with a Hill coefficient around 3.0. Both proteins can catalyze NO dioxygenation, and CYB5/CYB5R support this activity via rapid reduction of both globins. Overall, the functional characteristics of the zebrafish Cygbs seem similar to those of the human protein, suggesting very well-conserved roles for Cygb in vivo. These results support a conserved role for the CYB5/CYB5R/Cygb system through evolution, probably for NO regulation or NO detoxification.

Adjuvant Statin Therapy Efficacy is dictated by Tumor Dormancy and Statin Lipophilicity in ex vivo and in vivo Models of Metastatic Breast Cancer

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Metastasis in breast cancer patients heralds mortality, as disseminated disease is generally chemoresistant. After tumor cells reach the ectopic tissue, they undergo an epithelial reversion to enter a period of quiescence, termed dormancy, which may last for decades before outgrowing again as mesenchymal/dedifferentiated masses. Thus, long-term, relatively non-toxic interventions that prevent metastatic outgrowth are needed to treat this mortal stage of tumor progression.

Epidemiological analyses have suggested that statin usage, for cardiovascular indications, is correlated with a reduction in clinically-evident metastatic (though not in incidence of primary) breast cancer. The goal of this study is to demonstrate this is due to statins suppressing breast cancer cell proliferation and keeping the micrometastases in the dormant state.

We have found that atorvastatin and simvastatin limit the growth of some cancer cell lines, but not others. The sensitive lines were marked by lacking surface E-cadherin, the hallmark of the mesenchymal phenotype. When E-cadherin is downregulated on epithelial tumor cells, the cells become growth inhibited by the statins. Furthermore, this is a direct effect, as we now have shown that hydrophilic statins are relatively ineffective compared to the membrane permeant lipophilic statins as tumor cells generally lack the transporters that enable these drugs to gain access to the cells.

To determine whether the statins target the emergent metastatic tumor cells, we are using an all human microphysiological system (MPS) of the most common site for metastases, the liver. Briefly, a micro-hepatic tissue is established by seeding primary human liver cells in a porous scaffold subject to a physiological flow. RFP-labeled breast cancer cells are seeded into these microtissues and examined weeks later. Liver function and health are monitored by clinical chemistry assays performed on supernatant samples. We have previously shown that this system robustly reproduces tumor dormancy. Initial studies suggest that statins suppress the emergence of dormant tumor cells when challenged by stressors that lead to outgrowth. Additionally, atorvastatin suppresses proliferation of mesenchymal but not epithelial breast cancer cells in intrasplenic and mammary fat pad injection models for breast cancer metastasis to the liver and lung respectively. As 26% of adults currently take a statin for other medical conditions, these studies may suggest the best statin to use in the context of maintaining breast cancer dormancy long-term and delaying or avoiding the morbid emergence.

Engineering 2nd-Messenger Control Mechanisms for Cellular Programming

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In the field of tissue engineering, many tissue phenotypes are difficult to fully recapitulate in new engineered tissue constructs. In this work, we aim to expand the phenotypic range of engineered tissue via synthetic biology-inspired genetic modification of their cellular constituents. Using HEK-293 cells as a prototyping chassis, we are developing a calcium signaling pathway synthetic tookit containing optogenetic tools combined with components for interfacing with natural calcium signaling systems.

Calcium signaling controls a variety of cellular behaviors. Our goal is to controllably modulate calcium signaling in order to prescribe the subsequent phenotypic state of the cell. In order to tune calcium signaling behavior, we are developing genetic circuitry inspired by engineering control theory. We are developing both intracellular and extracellular mechanisms of feedback that can sense, and subsequently modify the calcium signaling behavior of the cells. Within the intracellular feedback loop, calcium sensing transcription factors (e.g., NFAT and NF-kB) will be recruited to drive gene expression for custom genetic circuitry designed to modulate calcium signaling behavior, thus creating a closed-loop control architecture.

An extracellular feedback architecture based on optogenetic calcium signaling proteins is also being developed. In order to sense the internal calcium state of the cell (i.e. intracellular calcium concentration over time), we are employing a range of genetically encoded calcium indicators such as GCaMP, and GECO, which respond fluorescently to calcium binding events. These molecules transduce calcium signals into light, which can then be processed by external hardware and software. To close the calcium signaling feedback loop, the cells are also transfected with optogenetic proteins designed to modulate calcium signaling. Opto-STIM1 is one such protein that modulates the activity of calcium release-activated calcium channels in response to light stimulation. With appropriate control structures, tunable feedback control of calcium signaling should be achievable by activating optogenetic calcium tools in response to fluorescently detected calcium signals.

Stem Cell Therapy Enriched Fat Grafting for the Reconstruction of Craniofacial Deficits

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PURPOSE: Fat grafting is an effective treatment for craniofacial deformities. Stromal vascular fraction (SVF) is a concentrated form of adipose derived stem cells (ASC) that can be isolated from fat through collagenase based procedures. The aim of this clinical trial is to assess the impact of SVF enrichment on craniofacial fat grafting.

METHODS: This IRB-approved prospective cohort study was funded by the Department of Defense. Twelve subjects with at least two regions of craniofacial volume deficit were enrolled and underwent fat grafting with SVF-enriched or standard fat grafting to each area. All patients had bilateral malar regions injected with SVF-enriched graft on one side and control standard fat grafting to the contralateral side. Outcome assessments included: 1) demographic information; 2) volume retention determined by CT scans; 3) SVF cell populations assessed by flow cytometry; 4) SVF cell viability; and, 5) complications. Follow-up was nine months.

RESULTS: All patients had subjective improvement in appearance. There were no serious adverse events. There was no significant difference in volume retention between the SVF-enriched and control regions overall (50.3% vs 57.3%, p=0.269) or comparing malar regions (51.4% vs 56.7%, p=0.494). Patient age, smoking status, obesity, and diagnosis of diabetes did not impact volume retention. Cell viability was 77.4±7.3%. Cellular subpopulations were 60.1±11.2% ASCs, 12.2±7.0% endothelial cells, and 9.2±4.4% pericytes. There was no significant correlation between cell viability or cellular subpopulations and volume retention.

CONCLUSIONS: Autologous fat transfer for reconstruction of craniofacial defects is effective and safe. SVF enrichment does not significantly impact volume retention.

Biofilm associated infections, inflammatory response, and melanoma progression

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Cellular therapy is an evolving treatment option for cancer. Appropriate activation and interaction of immune cells with each other and the tumor are implicit. This is particularly true for melanoma in which the clinical importance of the immune system has been recognized for decades. Ulceration of the primary cutaneous melanoma has been recognized as an important independent variable predicting poorer prognosis – a factor that has been incorporated into the current AJCC staging system. The reason for this effect remains unclear. We have recently demonstrated that bacterial colonization may occur at the site of ulceration often with the formation of a biofilm. Biofilms are active “communities” of microorganisms which reside within a protective extracellular polymeric substance (EPS). The behavior and persistence of biofilms is quite different from free floating microorganisms (planktonic). We have further demonstrated the presence of biofilm and associated microorganisms within the draining (sentinel) lymph node. This suggests the possibility the biofilm may alter the “normal” or usual antitumor response leading to tumor specific anergy, which may in turn impact or amplify the metastatic potential.

We have identified bacteria within 29 melanoma specimens recovered from the tumor banks within the AHN COE Biofilm Biobank. These specimens were analyzed using the PCR-MS-ESI-TOF technology. Additionally, fluorescence in situ hybridization (FISH) was used to visualize the bacteria within a subset of the specimens. 28 different bacterial species were identified from the tumor and lymph node specimens. In three subjects the bacteria isolated from the primary tumor was also recovered from the corresponding lymph node. The most prevalent bacteria detected were Propionibacterium acnes and Staphylococcus species. To further investigate the relationship between biofilm, metastasis, and inflammatory response, we used immunofluorescence to visualize general macrophage and pro-inflammatory N1 neutrophil presence on a subset of melanoma specimens.

Role of Calpain in Bicuspid Aortic Valve Aneurysmal Disease

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Introduction: Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation and is associated with aneurysm formation and thus, a heightened risk for dissection or rupture. We previously identified increased vulnerability to oxidative stress in smooth muscle cells (SMCs) in aortic specimens from BAV patients compared to specimens from patients with the morphologically normal tricuspid aortic valve (TAV). Other studies have shown that calpain is increased in tissue samples from patients with BAV compared to control. Lastly, it has been shown that inhibition of calpain reduces oxidative stress in vasculature. We hypothesize that oxidative stress stimulates calpain activity and increases downstream targets caspase 3 and matrix metalloproteinases (MMP) in BAV-associated aortopathy.

Methods: Ascending aortic SMCs were isolated using previously established protocols. Nearly confluent SMCs were cultured in serum-depleted medium for 48 hours. SMC populations isolated from BAV (n=9) and TAV (n=12) patients were then treated with 1000μM of tert-butyl hydroperoxide (tBHP) for 3 hours and then lysed. 5μg of protein from each lysate were transferred to each well of a 96-well black plate and incubated in 50μM of Suc-LLVY-AMC, a fluorogenic compound cleaved by calpain. For caspase 3 activity, BAV (n=13) and TAV (n=10) SMCs were treated with 100 and 1000μM of tBHP. After 3 hours, cells were lysed and incubated in 2mM of (Ac-DEVD)2-R110, a fluorogenic compound cleaved by caspase . BAV (n=9) and TAV (n=8) SMCs were treated with 1000μM tBHP ± 30μM of calpeptin, which inhibits calpain activity, for 3 hours followed by determination cell viability using an MTS-based assay. Finally, cultured BAV (n=11) and TAV (n=11) SMCs were treated with 100μM tBHP for 48 hours. MMP activity was measured in conditioned medium supernatant (2μg of protein) using a gelatin zymography technique.

Results: tBHP-induced calpain activity was higher in BAV SMCs (65±16.9%) compared to TAV SMCs (45.9 ± 5.9%), p=0.02. At 100μM and 1000μM tBHP, there was a higher increase in caspase 3 activity in BAV SMCs compared to TAV SMCs (2.5 ± 0.52 vs 1.3 ± 0.07-fold, p=0.016 and 3.2±0.69 vs. 1.4 ± 0.09-fold, p<0.01, respectively). Calpain inhibition rescued TAV SMCs from tBHP-induced cell death (66.4 ± 6.7% vs. 93.2 ± 9.3%, p=0.05) and did not affect BAV SMC viability (53.6 ± 6.8% vs. 66.3 ± 5.9% , p=0.34). Treatment with tBHP did not affect MMP activity in both cohorts. However, MMP at baseline and at 100μM tBHP were significantly higher in the BAV group compared to TAV (both p<0.01).

Conclusion: Calpain, a calcium dependent protease, is a proven inducer of caspase 3 and MMP. This current study demonstrates that tBHP induced calpain activity is increased in BAV SMCs and be responsible for the associated upregulation of caspase 3 and MMP activity. Future studies aim to elucidate this relationship between calpain and its downstream proteins. This study highlights a potential target that can be used to modulate SMC biology and extracellular matrix remodeling, that have been implicated in BAV disease.

Regulation of Oncogenic Signaling Pathways in Hepatocellular Carcinoma by the Pleiotropic Scaffold Protein IQGAP1

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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 to combat the disease are insufficient. Limited success has been observed with the current standard of practice by treating patients with the multi-kinase inhibitor Sorafenib. 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 of the underlying mechanisms that drive HCC oncogenesis is critical to develop effective methods of treatment. Recently, we characterized a mechanism in normal liver that results in polyploidy and is controlled by microRNA-122 (miR-122). miRNA-122 accounts for over 70% of total miRNAs in the liver. Approximately 50% of human HCCs are depleted of miR-122. MicroRNAs regulate the expression of multiple target genes, and we and others identified IQGAP1 as a direct target gene of miR-122. The scaffolding protein IQGAP1 regulates numerous signaling pathways associated with cellular proliferation (e.g., Wnt and EGFR pathways), while independently being implicated in hepatocarcinogenesisis. IQGAP1 expression is elevated in 60-85% of HCCs. To examine this relationship in liver cancer, we analyzed 10 human HCC samples for miR-122 and IQGAP1 expression. In our group, miR-122 was reduced in all samples and IQGAP1 was higher in 7 HCCs. We can replicate loss of miR-122 and elevated IQGAP1 by using a clinically relevant HCC tumor model that overexpresses mutant S45Y-beta-catenin/MET/IQGAP1. Our model shows higher levels of alpha-fetoprotein, a clinical marker of HCC, compared to beta-catenin/MET alone. Importantly, we identified for the first time that IQGAP1 interacts with the MET tyrosine kinase receptor. Together, our data suggests that IQGAP1 works with beta-catenin/MET to provide a clinically relevant model to explore whether restoring miR-122 expression can inhibit/reverse HCC tumorigenesis.

Structure-based virtual screening identifies small molecule inhibitor of the profilin1-actin interaction with anti-angiogenic properties

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Profilin1 (Pfn1) is an important regulator of the actin cytoskeleton and plays a vital role in many actin-based cellular processes. Previous studies have shown that profilin1's interaction with actin is important for endothelial cell (EC) migration and angiogenesis, suggesting that this interaction is a suitable target for an anti-angiogenic strategy. Based on an already resolved Pfn1:actin complex crystal structure, we performed structure-based virtual screening of small molecule libraries. We identified compounds that match the pharmacophore of the key actin residues of the Pfn1:actin interaction, and therefore have the potential to act as competitive inhibitors of this interaction. Subsequent biochemical assays identified two candidate compounds with nearly identical structures that can mitigate Pfn1's effect on actin polymerization in vitro. A proximity-ligation assay further confirmed compound-induced inhibition of the Pfn1:actin interaction in ECs. Treatment with these two compounds had potent anti-angiogenic effects on ECs both in vitro and ex vivo. Of note, consistent with the importance of Pfn1 in the regulation of actin polymerization, cell migration and proliferation, these compounds also reduced the overall level of cellular filamentous(F)-actin and slowed EC migration and proliferation. In summary, this study provides the first proof-of-principle of small molecule-mediated interference of Pfn1's interaction with actin, which may have potential utility for use in anti-angiogenic strategies.

Apical cell-cell adhesions reconcile symmetry and asymmetry in zebrafish neurulation

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The symmetric tissues and body plans of animals are paradoxically constructed with asymmetric cells. To understand how the yin-yang duality of symmetry and asymmetry are reconciled, we asked whether apical polarity proteins orchestrate the development of the mirror-symmetric zebrafish neural tube by hierarchically modulating apical cell-cell adhesions. We found that apical polarity proteins localize by a pioneer-intermediate-terminal order. Pioneer proteins establish the mirror symmetry of the neural rod by initiating two distinct types of apical adhesions: The parallel apical adhesions (PAAs) cohere cells of parallel orientation, and the novel opposing apical adhesions (OAAs) cohere cells of opposing orientation. Subsequently, intermediate proteins selectively augment the PAAs when the OAAs dissolve by endocytosis. Finally, terminal proteins are required to inflate the neural tube by generating osmotic pressure. Our findings suggest a general mechanism to construct mirror symmetric tissues: Tissue symmetry can be established by organizing asymmetric cells opposingly via adhesions.

Estrogen Receptor Expression in the Non-aneurysmal and Aneurysmal Human Thoracic Aorta

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Introduction
Thoracic aortic aneurysm (TAA), and its deadly sequelae of aortic dissection and rupture, may arise in the context of a normal, tricuspid aortic valve (TAV) or in patients with the congenital malformation bicuspid aortic valve (BAV). Though the pathogenesis of TAA has yet to be elucidated, clinical observations have revealed chronological distinctions between the sexes. One plausible explanation for these sex differences may be the influence of estrogen receptors (ERs) which have been implicated in other cardiovascular diseases. ER subtype (alpha and beta) expression in the human thoracic aorta has not been fully characterized and, furthermore, the role of ERs in TAA has yet to be determined.

Methods
RNA was isolated from ascending aortic media samples collected from surgical patients undergoing ascending aortic replacement for aneurysm and from heart transplant patients, with informed consent. RT-PCR was used to quantify relative expression of ESR1 (ER alpha) and ESR2 (ER beta). P values were calculated with a Mann-Whitney U test (SigmaPlot).

Results
Gene expression of ESR1 (n=36 females, n=41 males) and ESR2 (n=30 females, n=30 males) in the aortic media of non-aneurysmal and aneurysmal specimens did not differ significantly between the sexes. Furthermore, there was no significant difference in ER expression when comparing non-aneurysmal and aneurysmal patients among either valve morphology (TAV or BAV). ESR1 gene expression was higher than ESR2 gene expression across all groups. ESR1 gene expression demonstrated no correlation with age in either sex whereas ESR2 gene expression demonstrated a trend towards decreased expression with increasing age in males ($p=0.0529$) but not in females ($p=0.596$).

Discussion
Estrogen receptors alpha and beta are expressed in the ascending aortic media in both sexes and across all ages, indicating a persistence of these sex hormone targets despite low serum estrogen levels in men and post-menopausal women. ERs have been shown to regulate putative protective factors in TAA development, such as antioxidant enzymes, thus continued expression of ER genes may correlate with oxidative stress response in the thoracic aorta irrespective of circulating hormone levels. Further studies will be necessary to determine ER activity within the aortic media and to clarify the role of ERs in the pathophysiology of TAA formation and progression to dissection and/or rupture.

FIBROKINE(TM) Peptides: A Broad-Spectrum of Anti-Fibrotic Chemokine Peptides to Treat Organ Fibrosis

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Fibrotic diseases are associated with 45% of deaths in the U.S. but effective therapy does not exist, representing a major unmet medical need. Clinical and etiological manifestations of fibrotic disorders are diverse, yet they share underlying irritation that promotes the release of various growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines/chemokines. Persistence of this process causes excessive accumulation of extracellular matrix and chronic inflammation that alter the cellular and structural architecture of the tissue, decreasing its functionality. These changes can lead to organ dysfunction and death. Common fibrotic diseases including cardiac fibrosis, scleroderma, idiopathic pulmonary fibrosis, diabetic nephropathy, liver cirrhosis, rheumatoid arthritis, atherosclerosis, and nephritis share causative factors. Our group has designed and developed a class of chemokine-derived FIBROKINE(TM) peptides that successfully target the underlying causes of common fibrotic diseases and are capable of halting, reversing, and/or eliminating fibrosis or the underlying molecular processes causing fibrosis.

Chemokines are unique in their multifunctional roles and ubiquitous modulation of fibrotic processes. In this study, we test our newly generated class of chemokine-derived FIBROKINE(TM) peptides, developed using in-silico prediction-based functional peptide design. The efficacy of several FIBROKINE(TM) peptides was tested via functional assays for cell motility, proliferation, apoptosis, metabolic activity, angiogenic effects, and altered gene expression and protein profiles. Experimental systems evaluated primary human and murine dermal fibroblasts, endothelial cells, keratinocytes, cardiac fibroblasts, and cardiomyocytes, in both direct and indirect co-cultures as well as individually.

FIBROKINE(TM) peptides potently inhibited both chemotaxis and cellular function induced by pro-fibrotic chemokines. Specifically, FIBROKINE(TM) peptides reduced the expression of α-Smooth Muscle Actin and reduced mRNA and protein secretion levels of Collagen I, Laminin, Fibronectin, and Tenascin C in dermal and cardiac fibroblasts following induction by pro-fibrotic TGF-beta. FIBROKINE(TM) peptides also inhibited TGF-beta1-mediated activation of epithelial–mesenchymal transition in keratinocytes and dermal fibroblast co-cultures, a known feature of fibrosis. Lastly, FIBROKINE (TM) peptides significantly inhibited VEGF-induced endothelial motility and tube formation in vitro, properties critical for angiogenesis.

Our data suggest FIBROKINE(TM) peptides mimic biological activities of natural chemokines on fibrosis-inducing cell types. These data further reveal the effectiveness of the anti-fibrotic design of FIBROKINE(TM) peptides as novel, targeted therapeutic solutions capable of treating fibrotic conditions through disruption of multiple disease-causing mechanisms.

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Bi-directional Macrophage-Fibroblast Crosstalk Directs Wound Resolution Factors

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During wound repair, fibroblasts secrete abundant extracellular matrix (ECM) components and participate in remodeling of the provisional ECM. Macrophages and other immune cells also play an important role in all stages of wound healing. Classically activated macrophages (CAMs/M1) contribute to inflammation early on, whereas alternatively activated macrophages (AAMs/M2) secrete ECM-degrading enzymes including matrix metallopeptidases (MMPs) during resolution. Macrophages instruct fibroblast activities, largely via secreted factors. However, the differential effect of CAMs and AAMs on fibroblasts remains unknown. CXCR3 is a chemokine receptor that modulates activities of both fibroblasts and macrophages during wound healing. Activation of CXCR3 by its chemokine ligands is a critical stop signal that halts continuous ECM remodeling by fibroblasts. Yet, the importance of CXCR3 in macrophage-fibroblast crosstalk during tissue repair has not been explored. The goal of this study was to investigate the interplay between macrophages and fibroblasts and elucidate the role of CXCR3 signaling in their interaction.

We differentiated naïve macrophages (M0) into either CAMs/M1 using IFN-gamma and LPS, or AAMs/M2 using IL-4. To study the effects of secreted factors and cell-contact dependent factors, we used indirect and direct co-culture systems, respectively. The role of CXCR3 in macrophage-fibroblast cross talk was studied using cells from wild-type (WT) and CXCR3-/- knockout mice.

Cytokine array experiments revealed that fibroblasts treated with media conditioned by CAMs secreted higher levels of the CXCR3 ligands (CXCL10, CXCL11) than untreated fibroblasts. Because both fibroblasts and macrophages express CXCR3, it was unclear which cells might respond to these ligands. Further investigation using qRT-PCR, showed that treatment of WT and CXCR3-/- fibroblasts with media conditioned by wild-type CAMs induced expression of MMP8 and MMP9 compared to no treatment, suggesting that CXCR3 expression on fibroblasts is dispensable for CAM-mediated regulation.
Fibroblast induction of MMP8 and MMP9 was greatly diminished when fibroblasts were cultured with media from CXCR3-/- CAMs, indicating that this crosstalk response requires CXCR3 expression on CAMs. The influence of macrophage subtypes on MMP8, MMP9, and TGF-beta-induced alpha-smooth muscle actin and ECM protein production was observed by immunofluorescence staining.

Our data indicate that CAMs induce fibroblast expression of CXCR3 ligands and the key ECM-degrading enzymes MMP8 and MMP9. The expression of MMPs was independent of CXCR3 expression on fibroblasts, but required expression of CXCR3 on the CAMs. Thus, our study suggests, for the first time, that paracrine interaction between fibroblasts and macrophages dictate the effector functions of fibroblasts in wound healing.

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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 impaired bile flow, and there are few medical therapies available. Thyroid hormone T3 and synthetic thyroid hormone receptor agonists are known to cause induction of hepatocyte proliferation during liver regeneration through activation of beta-catenin. However, these drugs' therapeutic benefits in cholestatic liver disease is unknown. In this study, we administered GC-1, a thyromimetic that preferentially acts through the predominantly liver TR-beta receptor, to Mdr2 knockout (KO) mice, a commonly used murine model of sclerosing cholangitis. 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 on GC-1 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, Mrp2, Mrp3, Cyp2b10, and Oatp4, compared to KO mice on normal diet, with the net result being retention of BA in the hepatocyte. Interestingly, KO mice on GC-1 diet had decreased total and phosphorylated, active beta-catenin compared to KO mice on normal diet, suggesting an alternate mechanism for GC-1 activation in this model. 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 beta-catenin independent.

Regenerative Effects of Secretome Derived from Long-term Cryopreserved Stem Cells for Glaucoma Treatment

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Purpose: Stem Cell Therapy has revolutionized the area of regenerative medicine but has few side-effects. To minimize side-effects and maximize therapeutic efficacy, we hypothesize that secretome derived from long-term cryopreserved Stem Cells from the Trabecular Meshwork (TMSCs) can impart regenerative effects in glaucoma in vitro model and hence can offer an uninhibited approach for future clinical approaches.

Methods: TMSCs from four donors were thawed after 5-7 years of cryopreservation and assessed for expression of stem cell markers by real-time PCR and flow cytometry. Stem cell characteristics were evaluated for colony, spheroid formation and multipotency. Secretome was derived from the cultured cells during log phase. Regenerative effects of TMSC secretome were tested in dexamethasone induced in vitro cellular glaucoma model and wound healing. Differentiated TM cells treated with Dexamethasone with or without secretome were examined for expression of TM cell marker CHI3L1 and glaucoma-associated marker Myocilin. Wound healing was assessed using live-cell microscopy and qPCR for fibrotic gene expression. Statistical analysis was performed by ANOVA followed by Tukey post-test.

Results: Revived TMSCs expressed stem cell markers CD90, CD73, CD105, OCT4, KLF4, ABCG2. TMSCs maintained clonogenicity and spheroid-forming potential. They were able to differentiate into osteocytes with Alizarin red staining; neural cells expressing β-III Tubulin and Neurofilament; and TM cells expressing CHI3L1 and AQP1. Dex-treated TM cells with secretome regained expression of CHI3L1 and reduced expression of Myocilin. TMSC secretome enhanced the TM cell wound healing with increased migration and proliferation, and reduced the expression levels of fibrosis markers Fibronectin, CTGF and SPARC after wound.

Conclusion: This study provides evidence that secretome from long-term cryopreserved TMSCs has regenerative effects in vitro hence paving the way for further investigation in the field. Identification of individual proteins in secretome responsible for regeneration can greatly advance glaucoma treatment and have huge repercussions in regenerative medicine.

Cardioprotective Actions of Relaxin

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Background: Normal aging results in structural and electrical remodeling of the heart including fibrosis, inflammation and altered ionic currents, which reduce the threshold for cardiovascular disease development. Relaxin is a naturally occurring hormone of pregnancy that exhibits remarkable cardioprotective effects including anti-fibrotic and anti-arrhythmic properties. Despite the beneficial effects of relaxin, the mechanisms by which it mediates its anti-fibrotic and anti-arrhythmic effects remain largely unknown.
Methods: To determine if relaxin mediates its effects through regulation of gene transcription, RNA-sequencing was performed on young (9-months) and aged (24-months) F-344 male and female rat LV treated with control or relaxin for 14-days. Immunohistochemistry was used to confirm predictions based on transcription alterations. To elucidate the mechanism by which relaxin mediates its effect on sodium channel protein (Nav1.5) expression and collagen secretion, immunofluorescence was performed on isolated cardiomyocytes and fibroblasts, respectively.

Results: We show that aging resulted in significant alterations in inflammatory related gene expression in female but not male rats. Relaxin treatment resulted in a dramatic reversal of aged associated gene expression in females, and a suppression of inflammatory related genes in males. In addition, we show that relaxin increased Nav1.5 in isolated cardiomyocytes and increased nuclear β-catenin, indictive of canonical Wnt signaling activation. Further, DKK-1, a native inhibitor of canonical Wnt signaling, inhibited the effects of relaxin on Nav1.5 expression. Finally, we show that TGFβ mediated activation of fibroblasts resulted in significantly decreased expression of the canonical Wnts 1 and 3a, and the non-canonical Wnt7a, and relaxin reversed the effects on canonical Wnts only.

Conclusions: These data suggest relaxin as a potential therapy for inflammatory and fibrotic related cardiovascular diseases and determined a novel mechanism by which relaxin remodels the physical (fibrosis) and electrical (Nav1.5) cardiovascular substrate through interaction with canonical Wnt signaling.

Sub-additive effects of cell and physical therapy in a rodent model of stroke

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Stroke caused by the occlusion of a cerebral artery can lead to death or severe, long-lasting functional impairments. Treatment options, however, are extremely limited, extensive, and cost-intensive without the guarantee to restore lost functions. Physical therapy (PT) is known to also promote improvements in behavioral functions and the only treatment used to improve chronic behavioral impairments. Emerging approaches, such as stem-cell based therapies, are promising and can support tissue restoration and functional recovery by integration in the peri-infarct tissue. Although both approaches promote improvements in sensory and motor deficits, behavioral deficits remain. The aim of this study was to evaluate if the combination of human Neural Stem Cell (NSC) transplantation with physical therapy in a rat model of stroke will improve efficacy compared to either treatment alone. Adult male Sprague-Dawley rats underwent transient middle cerebral artery occlusion (MCAo). Success of MCAo was determined by T2-weighted magnetic resonance imaging (MRI) and animals were then randomly assigned to the following conditions: MCAo only, MCAo+NSCs, MCAO+PT, MCAO+NSCs+PT. Sham-operated animals served as healthy control. Groups subjected to NSCs or NSCs + PT received a peri-lesional NSC graft (450,000 cells) at 2 weeks post-stroke. Experimental groups with PT underwent daily treadmill running at a speed corresponding to 80% of their maximum capacity. Functional deficits and improvements were followed over a time course of 10 weeks using a battery of behavioral tests. Maximum capacity test (MCT, measures the maximum speed that the rats can maintain on a treadmill) showed that animals in the exercise group improved their performance by over 50%, while the untreated and control groups saw a decline (-10%). Bilateral asymmetry testing revealed that NSCs, exercise and combination therapy groups reduced the sensorimotor neglect by 20%, 33% and 42% compared to MCAo only. The foot-fault test measured animal's ability to integrate motor responses and demonstrated the most improvement in the NSCs treated group, with 57% fewer mistakes at week 10 compared to pre-transplantation. T2 and diffusion tensor imaging (DTI) MRI scans were acquired at the pre- and 10 week time point, including cerebral blood volume (CBV) and functional MRI (fMRI) scans in the concluding imaging session. T2-weighted MRI scans showed that the lesion volume did not change between any of the treatment or untreated groups. Immunohistological analysis of the graft revealed a good survival of the transplanted cells. A combination of physical and cell therapy therefore produces sub-additive therapeutic effect that are greater than each treatment by itself. Adjunct physical therapy is therefore an important factor to ensure optimal recovery after cell transplantation.

Exploring the Role of YAP1 in Liver Regeneration

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Chronic liver diseases cause long-term injury to the liver leading to fibrosis and eventually cirrhosis, for which the only treatment is a liver transplant. Notably, the liver has the unique ability to regenerate itself after injury, a complex process that depends on hepatocyte proliferation as well as genetic plasticity between the main epithelial cells of the liver, hepatocytes and biliary epithelial cells. Recently, the transcriptional coactivator Yes-associated protein 1 (YAP1) has been identified as a key regulator of liver tissue organization, liver size, cell proliferation, and genetic plasticity. While YAP1 is normally inactivated by the Hippo pathway and sequestered to the cytoplasm of mature hepatocytes, after various types of liver injury YAP1 is activated and translocated to the nucleus of hepatocytes, where it regulates transcription of various targets. It has notably been shown to promote biliary differentiation when it is experimentally over-activated in hepatocytes. Dysregulation of YAP1 activity has been implicated in numerous liver pathologies, but the precise functions of YAP1 in liver regeneration remain unclear.

Our goal is to investigate the role of YAP1 in the hepatocyte response to liver regeneration under various conditions. By treating adult YAP1loxp/loxp mice with hepato-tropic adeno-associated virus 8 (AAV8) carrying Cre-recombinase, we created an acute hepatocyte-specific YAP1 knockout model (AAV8-Cre YAP1 KO). We then subjected these mice to two types of liver injury: partial hepatectomy, which promotes hepatocyte proliferation to restore normal liver size, and the DDC diet model, which causes bile duct obstruction and stimulates hepatocytes to transdifferentiate into biliary epithelial cells. Here we show preliminary data exploring the role of YAP1 in these two models of liver regeneration. As expected, we observe a subtle decrease in proliferation in AAV8-Cre YAP1 KO compared to WT after partial hepatectomy. Interestingly, after treatment with DDC diet, there is a large selective pressure for YAP1-positive hepatocytes to replace YAP1-negative hepatocytes in the KO mice, suggesting that YAP1 plays an important role in the hepatocyte response to cholestatic injury. Further work is needed to clarify the source of YAP1-positive hepatocytes in this model and determine the role of YAP1 in transdifferentiation between hepatocytes and biliary epithelial cells after cholestatic injury.

Changes in neuronal activity are not correlated with vascular reactivity in APP-PS1 mice

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Introduction
Amyloid plaque load is an early pathophysiological hallmark of Alzheimer's disease (AD), occurring prior to significant neurodegeneration. Another common phenomenon observed in AD is cerebral amyloid angiopathy (CAA). The role of CAA in AD is not clear, but it might contribute to steepening decline. The long-term goal is to determine whether vascular reactivity is compromised in a mouse model of amyloidosis and whether it contributes to reduced metabolic supply with increases in neuronal activity.

Methods
APP-PS1 mice (B6C3.Tg-APPswe-PS1dE9, n=5) were obtained from MMRRC and injected with AAV-Syn-GCaMP6f virus in retrosplenial (RS) and somatosensory (SS) cortex. Head plate and cover glass were installed in the skull for awake imaging using a custom setup. Imaging sensitive to neuronal activity (GCaMP) and changes in blood oxygenation (OIS-BOLD) were acquired simultaneously in APP-PS1 as well as control mice (B6C3, n=2). Methoxy X-O4 was administered IP (1 mg/kg) to assess parenchymal and vascular amyloid plaque load.

Results
Vascular reactivity was assessed by CO2 administration and increases were observed in all mice, as expected. However, the increases in young APP-PS1 mice (3-4 months old) were larger than those of APP-PS1 old mice (12-14 months old; Table 1), indicating reductions in vascular reactivity with age. The variance in GCaMP signal was used as an index of neuronal activity. Its variance in RS regions were much lower in old vs. young APP-PS1 mice, which might result from increased plaque load in RS vs. SS in old AAP-PS1 mice. Its variance in SS was similar in both young and old APP-PS1 mice. These results suggest that reduced vascular reactivity is not a significant contributor to neurodegeneration since the SS neuronal activity was not significantly impaired with age.

Conclusion
Our findings thus far suggest that changes in neuronal activity are not correlated with vascular reactivity in APP-PS1 mice. This study is currently ongoing.

Shape Dependent Effects of Nanomaterials on Macrophage Polarization

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Macrophages display remarkable plasticity and polarize to different phenotypes in response to the local microenvironment. These changes give rise to different populations of cells viz. M1 (pro-inflammatory) and M2 (regenerative and wound healing) with distinct functions. In order to activate macrophages as a novel immunotherapeutic approach, current clinical trials and research strategies exploit lipopolysaccharide (LPS) or muramyldipeptide (MDP) or interferon-γ (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). However, in vivo toxicity of systemically administered LPS or rapid clearance and hence, short duration of action of MDP and IFN-γ has limited their transition as effective clinical alternatives. Nanoparticle (NP) research has recently shown great promise in modulating immune response by sustained delivery of cytokines. Excitingly, NP shapes can be engineered to modulate their uptake by macrophages, which in turn may affect macrophage activation. In this study, we test the hypothesis that NP shapes can be exploited to modulate the macrophage polarization towards a particular phenotype.

To achieve the goal of the study, we synthesized cerium oxide NPs (CNPs) in three different shapes viz. sphere, cube and rod, using hydrothermal synthesis method. We used human THP-I monocyte, an established model for macrophage differentiation. THP-1 cells are pre-differentiated to macrophages (M0) using Phorbol 12-myristate 13-acetate (PMA) and further polarized to M1 and M2 phenotypes using appropriate stimuli. Cytocompatibility studies revealed that the different shapes of CNPs were well tolerated by M0, M1 and M2 macrophages. We further measured the effect of 12h treatment of different CNP shapes at different doses (5-100 ug/mL) on driving macrophage polarization using expression of identified markers as readout. Sphere CNPs only maintained the phenotype of the pre-differentiated macrophages, but did not switch their phenotypes while Rod CNPs drove the pre-differentiated M0, M1 and M2 macrophages towards M1-like phenotype at all doses. Cube CNPs showed the ability to switch macrophage phenotypes (M1 towards M2 and M2 towards M1) at 25 ug/mL while favoring M1-like phenotype at 100 ug/mL doses for all three phenotypes. Since CNPs are also known for their ability to modulate reactive oxygen and reactive nitrogen species (ROS & RNS), we measured effect of CNP shapes on ROS/RNS levels in all three macrophage phenotypes. The cube CNPs markedly increased ROS/RNS levels compared to other shapes. Further studies are underway to determine if the observed effect of CNPs on macrophage polarization is due to the ROS/RNS levels or solely function of CNP shapes.

The data suggests that the shape of nanomaterial plays an important role in activation of innate immune response. These findings can be implemented to design better delivery systems for drugs targeting to the immune system.

Targeted translatome profiling using in utero gene transfer identifies ion channel pathophysiology in a brain development model of transcription factor 4 (TCF4) function

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The normal development of the brain results from a genetic program that is highly regulated and remarkably robust. Understanding the functional pathophysiology that results from the dysfunction of genes associated with mental health disorders can illuminate the key developmental aspects of generating and regenerating cells in the healthy brain. Transcription Factor 4 (TCF4) is a clinically pleiotropic gene associated with schizophrenia and the rare autism spectrum disorder (ASD) Pitt-Hopkins syndrome (PTHS). Contactin Associated Protein Like 2 (CNTNAP2) is one of the largest genes in the human genome and encodes a neurexin family protein also associated with schizophrenia and autism, as well as epilepsy, ADHD, and intellectual disability To gain insight about the neurobiology of TCF4, we created an in vivo model of PTHS by suppressing Tcf4 expression in rat prefrontal neurons immediately prior to neurogenesis. This cell-autonomous genetic insult attenuated neuronal spiking by increasing the afterhyperpolarization. At the molecular level, using a novel technique called iTRAP that combined in utero electroporation and translating ribosome affinity purification, we identified increased translation of two ion channel genes, Kcnq1 and Scn10a. These ion channels candidates were validated by pharmacological rescue and molecular phenocopy. Remarkably, similar excitability deficits were observed in prefrontal neurons from a Tcf4+/tr mouse model of PTHS. Thus, we identify TCF4 as a regulator of neuronal intrinsic excitability in part by repression of Kcnq1 and Scn10a and suggest that this molecular function may underlie pathophysiology associated with neuropsychiatric disorders. Our continued work on CNTNAP2 aims to investigate the hypothesis that this gene shares, with TCF4 and other ASD genes, downstream targets and common molecular pathways controlling critical aspects of normal brain development.

Nitrite Regulates Mitochondrial Dynamics to Inhibit Vascular Smooth Muscle Cell Proliferation

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Nitrite, a dietary constituent and endogenous signaling molecule previously thought to be a biologically inert product of endogenous nitric oxide oxidation, has recently been shown to regulate a myriad of biological processes. For example, nitrite has been shown to inhibit smooth muscle proliferation and attenuate restenosis after vascular injury. However, the mechanism of nitrite-dependent inhibition of smooth muscle proliferation remains elusive. The mitochondrion is a known subcellular target for nitrite, with physiological (micromolar) concentrations being shown to regulate mitochondrial morphology and function. Mitochondrial dynamics, the active formation (fusion) or fragmentation (fission) of cellular mitochondrial networks, has been previously shown to regulate cell cycle progression. Thus, we hypothesized that nitrite modulates mitochondrial dynamics and function to inhibit cell cycle progression and attenuate smooth muscle cell proliferation. Using rat aortic smooth muscle cells (RASMCs) we demonstrate that nitrite inhibits RASMC proliferation induced by platelet derived growth factor (PDGF) in a concentration dependent manner. This phenomenon is associated with mitochondrial fusion dependent on the upregulation of mitofusin-1 (Mfn1). Further, nitrite treatment upregulates the cyclin dependent kinase inhibitor p21, an effect that is abolished in Mfn1 deficient RASMCs. Ongoing studies are focused on determining the mechanism by which nitrite upregulates Mfn1 and stimulates mitochondrial fusion. These data have important implications for dietary and pharmacological modulation of vascular health and uncover a novel potential physiological mechanism for the regulation of smooth muscle cell number, especially in a clinical setting following percutaneous coronary intervention.

Lack of Beta-catenin in Hepatocytes Impairs Proliferation and Promotes Liver Progenitor Cell-Mediated Repair in Response to the Choline-Deficient Ethionine-Supplemented Diet

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Liver disease is the 12th leading cause of death in the United States. Treatments for chronic liver disease are limited due to incomplete understanding of liver regeneration mechanisms. When the normal mechanism of liver regeneration, 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 in mice via tamoxifen-inducible cytokeratin 19 (Krt19)-driven Cre recombinase to label BECs with tdTomato. In these mice we utilized anti-Ctnnb1 small interfering RNA (siRNA) conjugated to a hepatocyte-targeting ligand to knockdown expression of beta-catenin specifically in hepatocytes (KO3 mice). KO3 mice also displayed notably increased liver injury and significantly impaired hepatocyte proliferation after two weeks of CDE diet. After three weeks of CDE diet followed by two weeks of recovery we observed clusters of tdTomato-positive hepatocytes in KO3 mice, indicating BEC differentiation to hepatocytes. Thus, our results support the hypothesis that LPCs mediate liver regeneration when hepatocyte proliferation is impaired.

Age-Related Changes in Metabolism and Inflammation of the Liver

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Aging is associated with several molecular hallmarks: genomic instability, telomere attrition, mitochondrial dysfunction, stem cell exhaustion, and cellular senescence. The immune system is also prone to changes in function with age, including decreased adaptive immunity and increased inflammation. However, relatively little is known about the effects of aging on tissue-resident macrophages.

The liver contains the largest population of tissue-resident macrophages, known as Kupffer cells, which are maintained independently from the bone marrow into adulthood in the absence of liver injury. Alternatively. monocytes can traffic into the liver and become Kupffer cells if the niche is made available. It is unknown how the kinetics and functions of macrophage populations change with age in the liver.

To characterize the macrophage populations, cells were isolated from the livers of young (8-10 week) or aged (18-20 month) C57BL/6 mice (National Institute of Aging). Aged livers had an increase in total F4/80+ macrophages, as demonstrated by flow cytometry and immunofluorescent staining. The increase in macrophages was specifically due to an influx of CD11b+ expressing cells (F4/80+CD11b+CD68- and F4/80+CD11b+CD68+), which are thought to represent populations derived from circulating monocytes. There was no change in the number of F4/80+CD11b-CD68+ Kupffer cells with advanced age.

To identify mechanisms related to the influx of CD11b+ macrophages, potential liver pathologies were closely examined. There were no changes in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), bilirubin, and albumin. In addition, the aged mice did not show signs of glucose intolerance. Masson's Trichrome stain revealed no difference in liver fibrosis with age. However, H&E and Oil Red O staining showed a significant increase in fat deposits in the aged livers.

Aged murine livers showed a significant increase in triglyceride content, and were particularly enriched in oleic, palmitoleic, vaccenic, myristic, eicosapentaenoic, and linolenic fatty acids, suggesting a switch from glycolysis to lipogenesis with age. Furthermore, several chemokines, including monocyte chemoattractant protein (MCP-1) and members of the NF B pathway, were elevated at the RNA and protein level in hepatocytes isolated from aged livers.

Taken together the results demonstrate that aged livers develop steatosis (fatty liver) in the absence of dietary changes, and recruit CD11b+ bone marrow derived macrophages in response to elevated chemokine production. Future studies will examine functional changes in the macrophage populations to improve our understanding of age-related changes in metabolism and inflammation in the liver.

Improving mesenchymal stem cell therapeutic function through interaction with extracellular matrix protein Tenascin-C

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Human mesenchymal stem cells/multipotent stromal cells (MSCs) have already shown great promise in the wound healing arena through their secretion of regenerative paracrine factors, in addition to their ability to 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 that follows an injury. Previous work in our lab has discovered that when using an extracellular matrix (ECM) protein called Tenascin-C, we can bind the epidermal growth factor receptor (EGFR) and tether it to the plasma membrane. This prolonged sequestration of EGFR results in enhanced pro-survival signals, protecting MSCs against environmental pro-apoptotic signals. 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 in different phases of wound healing. With many of these gene targets being actively involved in ECM modulation and angiogenesis, we subsequently performed a series of 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.

Differences in Neural Stem Cell Identity and Differentiation Capacity Modulate Divergent Regenerative Outcomes in Lizards and Salamanders

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While lizards and salamanders both exhibit the ability to regenerate amputated tails, the regenerative outcomes achieved by each organism are remarkably different. Salamanders such as the axolotl (Ambystoma mexicanum) regenerate nearly perfect copies of original tails. Regenerated lizard tails, on the other hand, are “imperfect” copies of the originals. Some of the most important of these “imperfections” concerns dorsoventral patterning of regenerated skeletal and spinal cord tissues; regenerated salamander tail tissues exhibit dorsoventral patterning, while regrown lizard tissues do not. We observed that salamanders regenerate spinal cord ependyma with roof plate, floor plate, and lateral domains, while regenerated lizard spinal cord ependyma consist of floor plate only. Regenerated lizard tails also lack characteristically roof plate-derived structures such as dorsal root ganglia, and finally, salamanders, but not lizards, regenerate new spinal cord nerves. We hypothesized that differences in neural stem cells (NSCs) found in the ependyma of spinal cords regenerated by both lizards and salamanders account for these divergent regenerative outcomes. Through a combination of immunohistochemical staining, RT-PCR gene analysis, hedgehog regulation, and transcriptome analysis we analyzed NSC-dependent tail regeneration. Both salamander and lizard Sox2-positive NSCs form neurospheres in culture. While salamander neurospheres exhibit default roof plate identity, lizard neurospheres exhibit default floor plate. Hedgehog signaling in vitro and in vivo regulates dorsalization/ventralization of salamander, but not lizard, NSCs. Finally, examination of NSC differentiation potential in vitro shows that salamander NSCs are capable of neural differentiation into three lineages (neurons, oligodendrocytes, astrocytes), whereas lizard NSCs are not, which was confirmed by in vivo spinal cord transplantation. These findings suggest that NSCs in regenerated lizard and salamander spinal cords are distinctly different cell populations, and these differences contribute to the vastly different outcomes observed in tail regeneration.

Krüppel-like factor 4 (KLF4) Regulates Corneal Epithelial Stratification by Controlling Cell Polarity and Plane of Cell Division

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Asymmetry in cell division is important for tissue homeostasis, many adult stem cells balance self-renewal and differentiation by dividing asymmetrically. Cellular polarity is important in regulating asymmetric stem cell divisions in the cell and the cellular environment. Here we investigate the role of Yamanaka factor KLF4 in CE cell polarity and plane of cell division orientation, asymmetrical vs symmetrical.

Klf4 was ablated in the CE by feeding Klf4Δ/ΔCE (Klf4LoxP/LoxP /Krt12rtTA/rtTA/Tet-O-Cre) mice with doxycycline chow for at least a month. Littermates fed with normal chow served as controls. Expression of polarity markers and Rho family GTPases was examined by quantitative polymerase chain reaction (QPCR), immunofluorescent staining and immunoblots. Organization of actin bundles was examined using fluorescently tagged phalloidin. The pattern of immunofluorescent stain with anti-survivin and anti-phospho-histone 3 antibodies relative to the basement membrane was used to determine the plane of cell division as asymmetrical (0°-45°), or symmetrical (60°-90°).

Klf4Δ/ΔCE cells showed decreased expression and altered-localization of polarity markers; apical Pals1 and Crumbs1, apicolateral Par3, and basolateral Scribble. Cdc42 was upregulated, while Rac and Rho were mislocalized in the Klf4Δ/ΔCE cytoplasm unlike their membrane expression in the control. Phalloidin staining revealed disrupted actin cytoskeleton in the Klf4Δ/ΔCE cells. Survivin and phospho-histone H3 staining revealed a tilt in the Klf4Δ/ΔCE plane of cell division, with 61.4% symmetrical and 38.5% asymmetrical, compared with 36.8% symmetrical and 63.2% asymmetrical in the control.

Here we demonstrate the role of KLF4, in regulating CE polarity and plane of cell division, an important event for maintenance and expansion of stem cells. Further studies are needed to understand the involvement of KLF4 and its regulatory mechanisms important for governing self-renewal and differentiation, an important event in a successful stem cells therapy.

Intervertebral disc Degeneration in Duchenne Muscular Dystrophy

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Introduction: It is known that Duchenne muscle dystrophy (DMD) is a lethal muscle genetic disorder caused by the mutation of dystrophin gene. In addition to pathological degeneration of muscle & bone and depletion of muscle progenitor cells, intervertebral disc degeneration (IDD) was observed in the dystrophin/utrophin double knockout homozygous mouse (hom-dKO), a severe DMD model, but little is known about the molecular mechanism of disc abnormality. We tested the hypothesis that the IDD in the hom-dKO mice maybe caused by given signal pathways that results in IDD.

Methods: IACUC protocol was approved for all strains of animals used in this study (at least 4 samples per group) and the isolation of muscle and spine discs. Mice spine were isolated from wild-type (C57/BL10), mdx (dystrophin-/-), het-dKO (dystrophin-/-, utrophin+/-) and hom-dKO (dystrophin-/-, utrophin-/-) mice. Paraffin-embedded sections of all samples were stained with Hematoxylin and Eosin (H&E), Safranin O and Masson Trichrome staining. Total RNA was isolated from intervertebral discs for gene expression analysis by quantitative PCR to test the levels of matrix synthesis (Versican, Aggrecan), matrix proteases (MMP1, 13 and ADAMTS5) and inflammation environment (iNOS).

Results: 1) Spine discs of hom-dKO showed reduced extracellular matrix contents, cell number compared to other three groups. 2) Genes related to the synthesis of extracellular matrix, including versican and aggrecan, showed significant decrease while inflammation cytokines (iNOS) presented significant increase. However, the genes related to the degradation of matrix, MMP1, MMP13 and ADAMTS5, showed no significant changes (data not shown).

Discussion: This preliminary study explores the onset of IDD in DMD based on the histological analyses. And, results from quantitative q-PCR also demonstrate decreased matrix synthesis and increased inflammation during DMD IDD.

Conclusion: Spine disc in hom-dKO mice showed accelerated IDD and further characterization of the senescence-related pathway in IDD will be performed.

A role for polyploid hepatocytes in liver repopulation and adaptation to chronic injury

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A defining feature of the mammalian liver is polyploidy, a numerical change in the complement of chromosomes. Polyploidy affects up to 90% of hepatocytes in mice and 50% in humans. Diploid and polyploid hepatocytes proliferate during liver regeneration. However, polyploids often form multipolar spindles in mitosis, which lead to chromosome segregation errors and random aneuploidy. We hypothesize that ploidy subsets perform distinct roles in the liver: diploid hepatocytes are more proliferative, while polyploids facilitate liver adaptation or regeneration via aneuploid intermediates.

To study the differences between diploid and polyploid hepatocytes we utilized liver-specific knockout mice lacking transcription factors E2f7 and E2f8 (E2f7/E2f8-lko), as they are enriched for diploid hepatocytes. First, our FACS analyses showed E2f7/E2f8-lko mice contained 10 times more diploid hepatocytes and up to 10-fold fewer polyploids, which is in agreement with previous studies. Secondly, we tested how loss of polyploidy affected cell proliferation. Our in vitro assays showed young and adult E2f7/E2f8-lko hepatocytes had increased proliferation compared to controls. To measure proliferation in vivo we mixed E2f7/E2f8-lko and control hepatocytes in defined ratios, transplanted them into Fah-/- recipients, and determined the contribution of each genotype in the repopulated liver. Following repopulation, E2f7/E2f8-lko hepatocytes dramatically outcompeted the controls. These data indicated ploidy and gene expression differences were affecting cell proliferation. Finally, we examined aneuploidy in the control and E2f7/E2f8-lko mice. Control livers were 50% aneuploid, but only 7.5% of E2f7/E2f8-lko hepatocytes were aneuploid.

In conclusion, we showed that E2f7/E2f8-lko livers are enriched in diploid hepatocytes and almost completely euploid. The data demonstrate that diploid hepatocytes in E2f7/E2f8-lko mice are more proliferative than control polyploid hepatocytes, and polyploid hepatocytes are needed for the generation of aneuploid hepatocytes. Future work will determine the abilities of E2f7/E2f8-lko livers, which are predominantly diploid and euploid, to adapt to chronic liver injury.

Patient-Derived Valvular Interstitial Cells Model Reveals the Therapeutic Effect of Shape-Specific Nanoceria against Oxidative Stress Induced-Calcification

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Lack of effective pharmacological treatment makes valvular calcification a significant clinical problem. Elevated oxidative stress have been identified as a prominent driving factor for valvular calcification. Human valvular interstitial cells (hVICs) are known to mediate valvular calcification. In this study, we first developed a hVIC calcification model induced by elevated oxidative stress. Meanwhile, antioxidant nanoparticle based therapeutic such as cerium oxide nanoparticles (CNPs) offer an alternative to current treatments that have failed to stop calcification progression. Therefore, we then investigated the uptake profiles of shape-specific CNPs in hVICs, their antioxidant properties, and anti-calcification effects in the established calcification model as a function of treatment time (early vs late stage treatment).

Of different calcification stimuli tested, seven-day treatment of phosphate monobasic alone induced hVIC calcification as evidenced by Alizarin Red S (ARS) staining and calcium content. The addition of H2O2 further promoted calcification. Such in vitro calcification model recapitulates the proper cellular component (stenotic hVICs) and biochemical cues (elevated oxidative stress and hyperphosphatemia) in valve calcification.

We demonstrated that all CNPs tested can be readily taken up by hVICs as early as 1 h. Sphere and cube CNPs have significant higher cellular uptake than rod CNP. Macropinocytosis played a role in mediating the endocytosis of all CNPs. In addition, endocytosis of cube CNP were also clathrin-mediated and that of rod CNP were caveolae-and clathrin-mediated. Among three shape-specific CNPs, rod CNP was the most efficient in sustaining the antioxidant effect for at least three consecutive H2O2 challenges measured by AmplexRed® assay. However, butylated hydroxytoluene (BHT), a standard antioxidant, could not sustain the antioxidant effect. Given at different onsets (day 1 and day 4 as early vs late stage treatment), rod CNP inhibited calcification as demonstrated by reduced ARS staining, calcium content and mRNA expressions of osteoblastic markers (RUNX-2 and OPN). CNP treatment also increased the antioxidant enzyme activities of hVICs.

In conclusion, we first established an in vitro hVIC based calcification model induced by oxidative stress. We further demonstrated that different shape-specific CNPs can be taken up by hVICs and the shape of nanoparticle play an important role in determining their uptake profiles. CNP treatment, given at either early or late stage treatment, mitigated the calcification progression.

Analysis of Shear-Induced Erythrocyte Deformation Following Intracellular Content Replacement

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We have developed a method of removing erythrocyte intracellular contents and subsequently replacing them with high concentrations of exogenous protein. While this platform technology has a variety of potential applications such as encapsulation of pharmaceuticals for conversion of erythrocytes into intravascular delivery vehicles, our primary focus has been on development of treatments for hemoglobinopathies, most notably sickle cell disease. In order to be viable in circulation it is critical that the modified erythrocytes maintain the ability to deform under physiological shear stress, as membrane damage associated rigidification will prevent perfusion of the microvasculature and result in premature removal by the reticulo-endothelial system. In this poster we present proof-of-concept results of hemoglobin replacement using healthy bovine erythrocytes. Following partial hemoglobin replacement, modified erythrocytes were analyzed using rheological methods. Modified erythrocyte deformability was analyzed by a Linkam shearing stage device, with elongation index quantification performed using Image J. Viscosity was measured using Brookfield cone and plate viscometer. Preliminary results indicate relatively trivial changes in these rheological parameters following erythrocyte intracellular content replacement.

Elucidating the roles of cenpf and foxm1 in 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 their proliferative capacity significantly decreases as adults. Failure to replace damaged tissue with new CMs increases scarring and morbidity. Therefore, it is critical to discover which mechanisms regulate CM proliferation. Zebrafish offer insights into this because their CMs robustly proliferate after adult injury and have conserved homology with mammals; this allows 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. We identified two genes, cenpf and foxm1, that were upregulated during CM proliferation. Cenpf, a kinetochore-binding protein, and Foxm1, a forkhead-binding transcription factor, are involved in cell division and are expressed in mammalian cardiac development, but their roles in cardiac regeneration and subsequent CM proliferation have not been characterized. We hypothesize that Cenpf and Foxm1 are critical for CM proliferation, and loss of their expression will promote increased scarring and delayed cardiac regeneration.

To test this, we acquired ENU-derived mutant zebrafish lines foxm1-SA10708(foxm1-/-) and cenpf-SA12296 (cenpf-/-) from ZFIN. We removed 20% of the ventricle via amputation, and extracted hearts at specific days post-amputation (dpa) to observe changes in CM proliferation (3dpa and 7dpa) and fibrosis (30dpa) using immunofluorescent (IF) imaging and AFOG staining, respectively. We found at 30dpa cenpf-/- hearts displayed significantly increased scar area compared to WT hearts. Using IF, we identified that Cenpf strongly co-localized in CMs at 3dpa in WT hearts compared to other cell types. IF for CM proliferation displayed no significant difference between WT and cenpf-/- hearts at 7dpa. Interestingly, foxm1-/- hearts displayed significantly decreased levels of CM proliferation at 7dpa compared to WT and we are elucidating if they also show scarring similar to cenpf-/- hearts at 30dpa. These preliminary findings indicate that Cenpf and Foxm1 are necessary for CM proliferation after injury, and future experiments will be performed to understood the exact mechanisms they act through to promote proliferation.

The aorta and vocal fold paralysis: is there a connection?

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The vocal folds play a key role in the protection of the airway during swallowing, regulation of breathing, and voice production. Unilateral vocal fold paralysis (UVP) occurs when the recurrent laryngeal nerve (RLN) is damaged. The RLN innervates all of the muscles of the larynx except for the cricothyroid muscles. The left RLN bifurcates from the vagus nerve in the thorax near the aortic arch and courses adjacent to the underside of the aorta before ascending toward the larynx within the tracheoesophageal groove (1-3). Idiopathic UVP is a challenging condition where the cause of nerve functional loss is unknown (4, 5). Several case-based studies suggest that the extended course of the left RLN through the thoracic cavity potentially expose the left RLN to damaging forces associated with compression and stretch from adjacent pulsating anatomical structures including the pulmonary arteries, aorta, and left atrium (6).

The purpose of this study was to compare gated MRI derived aortic arch diameter and compliance between individuals diagnosed with left-sided idiopathic UVP and age- and gender-matched controls. Twenty participants met inclusion criteria and there were 10 in each group (idiopathic UVP and control). The mean compliance ($p=0.002$) and aortic arch diameter change ($p=0.04$) of the iUVP group was shown to be significantly higher than the control group within the same age group. A Tukey's procedure showed compliance among all age group are significantly different as well ($p<0.005$). The findings of this study supported the hypothesis that changes in aortic compliance may be a factor in the onset of left-sided idiopathic UVP. Future work in our laboratory is focused on investigating to what extent aortic thickness and material (constitutive) properties contribute to the compliance differences we observed in idiopathic UVP patients.

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Generation of mixture models to predict colloidal properties of triphasic nanoemulsions designed for neuroregeneration support in peripheral nerve injury

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There is a current need for novel, noninvasive imaging strategies in the field of peripheral nerve injury and regeneration (Janjic and Gorantla 2017). We postulate the complex triphasic perfluorocarbon (PFC)/hydrocarbon (HC)/water triphasic nanoemulsions (NEs), developed in our group (Patel et al, Plos One 2013, Janjic et al, Biomaterials 2014), can be engineered for use in this field of need using quality by design methodologies. On multiple occasions, PFC NEs have shown efficacy in visualizing neuropathic pain in animals (Weise, Basse-Luesebrink et al. 2011, Vasudeva, Andersen et al. 2014). Clinical success, which is a major challenge (Satalkar, Elger et al. 2016), requires nanomedicine formulations to exhibit adequate shelf-life and consistent quality attributes. Nanoparticle shelf-life and in vivo performance are sensitive to droplet size and size distribution of the formulation (Desai 2012). Therefore, it is critical to fully understand nanoparticle dependence on composition. One strategy for obtaining consistent quality is to build critical properties into the product during the development stage (Duncan and Gaspar 2011). Our PFC/HC/W nanoemulsions have previously been developed to allow therapeutic and diagnostic functions to occur simultaneously (Patel et al, Clinical Immunology 2015 and Janjic, Vasudeva et al, J. Neuroimmunology 2015). Here, we have applied mixture modeling to predict size and size distribution to a similar set of unique formulations. The models aim to contribute to understanding of individual component contributions of complex nanoemulsions, thereby providing a link between composition and performance. Four PFC and hydrocarbon oils exhibiting diverse structural properties as well as one solubilizer and a HC-PFC conjugate were selected as model excipients. NE size and size distribution models were fit and validated with new samples. This innovative characterization marks a first-of-its-kind approach to formulating and characterizing complex PFC NEs.

An Adaptive, Negative Feedback Circuit in a Biohybrid Device Reprograms Dynamic Inflammation Networks In Vivo

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Acute inflammation accompanies and underlies the pathobiology of sepsis, but is also central to tissue healing. We demonstrated previously the in vivo feasibility of modulating the central inflammatory mediator tumor necrosis factor-alpha (TNF-α) through the constitutive production of soluble TNF-α receptor (sTNFR). We have now created multiple, stably transfected human HepG2 cell line variants expressing the mouse NF-κB/sTNFR. In vitro, these cell lines vary with regard to baseline production of sTNFR, but all have ~ 3.5 fold elevations of sTNFR in response to TNF-α. Both constitutive and TNF-α-inducible sTNFR constructs, seeded into multi-compartment capillary-membrane liver bioreactors, could reprogram dynamic networks of inflammation and modulate key physiological outcomes in both endotoxemic and septic rats. Thus, control of TNF-α may drive a new generation of tunable biohybrid devices for the rational reprogramming of acute inflammation.

Computationally optimizing the compliance of biopolymer tissue engineered vascular grafts

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Cardiovascular disease (CVD) is the most common underlying cause of death. Coronary artery bypass grafting (CABG) remains one of the main intervention therapies for CVD. Tissue engineered vascular grafts (TEVGs) offer an alternative to existing graft options which often fail due to compliance mismatch amongst other reasons. In this study, we used an experimental/computational optimization method to develop and fabricate biopolymer TEVGs compliance-matched to rat aorta.

In an effort to mimic the alternating collagen/elastin layered geometry of native aorta, the TEVGs were made of alternating layers of crosslinked electrospun porcine gelatin and human tropoelastin. Each biopolymer layer was mechanically characterized individually at different crosslinking times using an in-house optomechanical biaxial tensile testing device [1]. The generated stress-strain data were used to develop a predictive model which were used as part of an optimization routine that was used to determine crosslinking duration, number of layers, individual layer thickness of a TEVG that would compliance match rat aorta [2]. The scheme was iterated until the finite elements model compliance-matched the target compliance. The optimized parameters were then validated experimentally by comparing the experimental compliance to the predicted value.

Our research group has successfully predicted the compliance of single layered gelatin constructs with a relative difference of 9.2% [2]. For this study, the target compliance was 0.00071 mmHg^-1. The optimized gelatin/tropoelastin layered constructs were found to have an experimental compliance 0.0004 ± 0.0002 mmHg^-1. Future studies will investigate modulating the compliance of individual layers by adding synthetic polymers like polycaprolactone, which could provide more accuracy in predicting compliance values of layered TEVGs to compliance-match native arteries.

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A Single Nucleotide Polymorphism in MPPED2 is Associated with Initial Hypo-Inflammation and Adverse Clinical Outcomes after Trauma

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Background: Major trauma is positively correlated with altered systemic inflammation, increasing rates of complications such as multiple organ dysfunction, prolonged hospitalization and increased mortality. Extensive advances in understanding of, and data, on the human genome has led to the hypothesis that genetic variability can drive outcomes following injury. Recently, we identified rs2065418, a single nucleotide polymorphism (SNP) in the metallophosphoesterase domain containing 2 (MPPED2) gene, in a set of 7 SNPs that are associated with an altered inflammatory response and non-survival after trauma. Herein, we sought to identify a key SNP among the 7 SNPs and investigate its association with altered clinical outcomes, organ function, and systemic inflammation in severely injured blunt trauma survivors.

Methods and Main Results: We identified MPPED2 rs2065418 as a key SNP, and investigated its association with outcomes and inflammation as a function of injury severity. Severely injured patients (Injury Severity Score ≥ 25) carrying the rs2065418 AA genotype exhibited longer hospital length of stay (LOS; 23 ± 2 d), ICU LOS (15 ± 2 d), a greater requirement for mechanical ventilation (DOV; 10 ± 2 d), higher creatinine plasma levels, and higher Marshall MODScore over 7 days vs. the control group of rs2065418 AB/BB high-severity patients (LOS: 16 ± 1 d, $p = 0.002$; ICU LOS: 10 ± 1 d, $p = 0.03$; DOV: 6 ± 1 d, $p = 0.007$; plasma creatinine; $p < 0.0001$ MODScore: $p = 0.0001$). Furthermore, high-severity rs2065418 AA patients had significantly different plasma levels of 11 circulating inflammatory mediators and reduced dynamic network complexity vs. controls.

Conclusions: The rs2065418 AA genotype in the MPPED2 gene is associated with hypo-inflammation, organ dysfunction, and greater resource utilization. A screening for this specific SNP at admission might stratify severely injured patients regarding their lung and kidney function and clinical complications.

Wearable pulse oximetry with soft 3D printed sensor and electronics for detecting perfusion anomalies through continuous monitoring

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In this work, we develop a patient-specific, 3D soft sensor that is subsequently integrated with flexible electronics to develop a wearable pulse oximeter for continuous biomonitoring. Pulse oximetry is a diagnostic tool that monitors blood oxygen saturation and can detect anomalies in vessel blood volume through continuous monitoring. These anomalies are initially asymptomatic because of the body's compensatory mechanisms for partial vessel obstruction and can lead to peripheral arterial disease. Freeform reversible embedding (FRE) is a 3D printing technique developed by the Feinberg lab to build liquid structures from the bottom up, which is impossible using traditional methods. The technique relies on a sacrificial hydrogel bath to support the layering of silicone prepolymers that are gel-like into a sensor that is soft and matches the elastic properties of biological tissue. Patient scans are used to develop an anatomically-conforming computer aided design (CAD) model of the sensor that is digitally adjustable for 3D printing. Combining the anthropomorphic sensor with soft electronics for pulse oximetry produces a biosignal monitoring device that can be tailored for comfort and to optimize the signal to noise ratio. Our wearable pulse oximetry is promising for continuous monitoring and an alternative to commercially available pulse oximeters are traditionally made from rigid materials.

Hemodynamic Changes during LVAD implantation may be associated with Subsequent Right Ventricular Failure: Opening the Black Box of the Operating Room

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Background: Approximately 20% of LVAD recipients experience acute RV Failure (RVF) post-implant. Studies have investigated hemodynamic parameters at the pre- and post-operative phase of LVAD therapy; none have looked at hemodynamics during implant. In this pilot study, we collected hemodynamic data during various stages of implantation to determine if changes in values can determine outcomes.

Methods: Pulmonary arterial (PA) waveform printouts or screenshots, acquired via Swann-Ganz catheter, were obtained at 5 stages of LVAD implantation: T(-2) Pre-operative with conscious patient in catheterization lab 9 ± 11 days pre-op, T (-1) Perioperative with patient under anesthesia pre-sternotomy, T(0) Chest open with LVAD on, T(1) Chest closure with LVAD on, and T(2) In the ICU 4- 24 hrs post- chest closure. Custom MATLAB scripts re-digitized the captures and generated an average representative waveform at each stage. PA pulsatility index (PAPI) and CVP/PA DBP were also calculated. Rate of hemodynamic change from LVAD on to post-op in ICU (T(-1) to T(2)) was calculated with mixed effect models. Association between RVF and baseline values as well as rate of change was calculated using Odds ratio.

Results: Data were obtained for 32 patients. Five patients experienced RVF defined as >14 days post-operative inotropic support (21 male, median age (IRQ) 55 (41-66) vs 4 male, age 59 (53-65) for no RVF and RVF, respectively). As previously reported, baseline RAP and RAP/PCWP were associated with RVF. No changes reached statistical significance; however, smaller decreases in PA DBP, mPAP, and CVP from T(-1) to T(2) showed trend of association with RVF (Odds ratios 1.34 ($p = 0.12$), 1.43 ($p = 0.13$), and 1.22 ($p = 0.17$), respectively).

Conclusion: Preliminary data indicate that there may be important hemodynamic changes during implantation which may be associated with a patient's odds of subsequent RVF. A larger sample size is required to confirm these findings.

Biomechanical Analysis Of Complications In Head Immobilization Devices For Pediatric Neurosurgery

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Precise and firm fixation of the cranium is critical during craniotomy and delicate brain neurosurgery making head immobilization devices (HIDs) a staple instrument in brain neurosurgical operations today. However, despite their popularity, now standard procedures exist for their use and many complications arise from HID use. In this paper, we identify biomechanical causes of complications and quantify risks in pin-type HIDs like clamping force selection, positioning and age effects. Based on our root cause analysis, we develop a framework to address the biomechanical factors that influence complications and understand the biomechanics of the clamping process. We develop an age dependent finite element model (FEM) of a single pin on a cranial bone disc with the representative properties and skull thickness depending on age. This model is utilized to reduce risk of complications and for pin design and to provide recommendations for current practices.

Development of a Combined Artificial Lung and Kidney

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The American Lung Association reports nearly 350,000 deaths each year due to some form of lung disease. Extracorporeal membrane oxygenation (ECMO) is commonly used to provide respiratory support to lung failure patients. According to the Extracorporeal Life Support Organization report, 87,366 patients were in need of ECMO treatment between 1990 and 2017. Patients on ECMO often develop impaired renal function and require renal replacement therapy (RRT). Irregular hemodynamics, systemic inflammation, and organ crosstalk present during ECMO can cause significant renal dysfunction. The continuous flow generated by ECMO may not be sufficient to maintain adequate tissue perfusion and oxygen delivery in peripheral organs such as the kidney. Systemic inflammation and acute changes in myocardial physiology often present during ECMO can also contribute to kidney dysfunction. Moreover, ECMO treatment can cause blood damage (hemolysis) and result in elevated plasma free hemoglobin which is toxic for kidney function.
More than 75% of ECMO patients develop acute kidney injury and RRT is required in approximately 50% of these patients. The risk of mortality in patients receiving simultaneous ECMO and RRT is nearly twice that of ECMO alone. Combined ECMO and RRT therapy requires consideration of how best to interface the two circuits. As recently reported in an international survey of 65 ECMO centers, 50.8% of centers use a RRT machine while 21.5% use only an in-line hemofilter. This demonstrates a surprising lack of standardized treatment methods for this patient group.

The current work aims to develop a compact and integrated device capable of both respiratory support as well as RRT. A device specifically optimized for combined ECMO-RRT therapy should be able to reduce circuit size/volume as well as device induced hemolysis. It is our hope that such a device will enable improved and more standardized treatment methods for this patient group and ultimately reduce the associated mortality.

Triple Functionalization of Titanium Aluminum Vanadium

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Orthopedic implant procedures are on the rise in the United States due to an aging population. At least a million of these procedures are performed annually. Common materials used for these applications include titanium and its alloy, titanium aluminum vanadium (Ti-6Al-4V). These implants can suffer from aseptic loosening and bacterial colonization, necessitating removal and replacement, which is very taxing on the patient. The goal of this work is to use self-assembled monolayers (SAMs) as linkers to immobilize multiple bioactive molecules to help prevent bacterial adhesion to and aseptic loosening of Ti-6Al-4V implants. In this work, phosphonic acid monolayers with different functional tails were formed on Ti-6Al-4V. These SAMs were then used as linkers for the immobilization of bioactive molecules, such as vancomycin. Diffuse reflectance infrared Fourier transform spectroscopy was employed to determine film stability as well as successful attachment of bioactive molecules.

Co-administration of Sodium Nitrite during Ultrasound Targeted Microbubble Cavitation Therapy Enhances Nitric Oxide Generation and Increases Microvascular Perfusion

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Percutaneous coronary intervention (PCI) is the mainstay therapy for recanalization of coronary arteries and restoring myocardial perfusion. Despite successful PCI, many patients suffer from failure of microvascular perfusion known as microvascular obstruction (MVO). MVO results from microvascular spasm, ischemia-reperfusion injury, and thrombotic occlusion. We have demonstrated reperfusion efficacy of ultrasound-targeted microbubble cavitation (UTMC) therapy in MVO, resulting in both mechanical dissolution of obstructing thrombi and upregulation of nitric oxide (NO). Previous literature shows that nitrite therapy has significant cardioprotective effects in MVO, while enhancing vascular bioavailability of NO. Accordingly, we sought to determine whether co-administration of nitrite could enhance efficacy of UTMC therapy.

Long pulse ultrasound was applied to rat hindlimb for 2 minutes during intra-femoral infusion of lipid microbubbles. Sodium nitrite (4 mg/kg) was administered via a pre-treatment bolus (confirmed 100 uM). After UTMC therapy, burst-reperfusion contrast ultrasound imaging was performed over 30 minutes. Ultrasound image intensities were measured in the treatment region to obtain microvascular blood flow. An NO catheter probe was placed in the gastrocnemius of the treated hindlimb to measure real-time NO concentration.

There was a continuous significant decrease in NO concentration over 30 min for UTMC-only ($n = 4$) and nitrite-only ($n = 3$), while UTMC+nitrite ($n = 5$) sustained significantly higher NO concentration over time ($p < 0.0001$). UTMC+nitrite had significantly higher blood flow than UTMC-only at all time points except 6 minutes ($p < 0.05$). At 3 minutes post-treatment, the UTMC-only group showed a 2.5-fold decrease in blood flow from baseline ($p < 0.05$), indicating a transient microvascular vasospasm not present in the UTMC+nitrite group.

These results show that nitrite co-administration during UTMC therapy enhances NO concentration and increases microvascular perfusion. In addition, the previously observed transient microvascular spasm during UTMC-only did not occur during nitrite co-administration, suggesting improvement of UTMC therapeutic efficacy.

Potential rheological treatment of Sickle Cell Disease (SCD) by reducing vaso-occlusion in small vessels

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It is well known that deformable RBCs tend to move towards the centerline of smaller vessels, leaving less deformable cells (platelets, leukocytes, etc.) near the vessel wall (forming the “cell free layer”). Discovered almost 100 years ago by Robin Fåhraeus, this phenomenon is now known as the Fåhraeus Effect.

Within blood vessels of patients suffering from SCD, rigid deoxygenated sickled RBCs (SRBCs) are mostly located near the vessel walls due to the Fåhraeus effect. This leads to an increased number of SRBCs traveling into vessel branches and contributes to the blockage of these vessels by SRBC-leukocyte-endothelium interactions leading to vaso-occlusive pain crises for patients.

Previously, we have showed that certain water-soluble non-toxic long-chain polymers added to blood significantly reduce or eliminate the cell free layer causing RBCs to become evenly distributed across the entire vessel diameter. In SCD patients’ blood, this effect may potentially reduce the number of SRBCs entering vessel branches while significantly increasing the number of normal healthy RBCs. Consequently, we hypothesize that this effect would also decrease the frequency of vaso-occlusion in SCD patients. To confirm our hypothesis, we have designed and built branched microfluidic devices to test the effects of these polymers added to mixtures of normal and rigid RBCs to prove the potential reduction of rigid RBCs entering vessel branches and their shunting through larger vessels instead. Our earliest experiments demonstrated significantly fewer rigid (sickled) RBCs entering branches, with a greater number of healthy RBCs entering branches as compared to control samples (without polymer).

Following these studies, tests will be carried out in microfluidic devices with multiple bifurcations to more closely resemble the microvascular tree and to demonstrate how additional generations of bifurcations may compound the effects shown in our preliminary studies. Future work will pursue replication of these results with intravital imaging.

Acute In Vivo Functional Assessment of a Novel Bioinspired Scaffold-Based Engineered Heart Valve with Biodegradable Magnesium Alloy Stent

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Objective: To evaluate the acute in vivo function of a novel, bioinspired scaffold-based tissue engineered heart valve (TEHV) with a biodegradable metallic stent.

Methods: Tri-leaflet pulmonary valves were fabricated by electrospinning poly(carbonate urethane)urea (PCUU) using a double component fiber deposition technique. The valve was then fastened to a biodegradable stent made of magnesium alloy AZ 31. Yorkshire pigs underwent median sternotomy, cardiopulmonary bypass, and stented TEHV implantation in the pulmonary position. Epicardial echocardiography was performed after weaning from bypass. Animals underwent repeat echo and explant analysis at either a humane endpoint or 12 hours. The TEHV underwent gross inspection and scanning electron microscopy (SEM) for structural analysis. Leaflets also were examined with histology and biaxial mechanical testing.

Results: Five animals underwent valve implantation, with 4 animals successfully weaned from cardiopulmonary bypass surviving between 2 and 12 hours. No animal deaths were found to be related to valve complications on necropsy. Echocardiography revealed good valve leaflet motion with no regurgitation, with an average peak velocity of 2.5 m/s through the pulmonary valve orifice demonstrating good flow. Repeat echo at the 12-hour time point was unchanged from post-operative measurements. SEM showed early signs of surface degradation of the magnesium stent. SEM of the TEHV leaflet showed retained microstructural architecture with no significant platelet activation. On two of the 5 explanted stents, there was evidence of fibrin sheath formation on the stent body, but not on the TEHV. Histology confirmed no significant cellular uptake by the TEHV. Biaxial stress testing revealed retained mechanics of TEHV fibers.

Conclusions: A bioinspired scaffold-based TEHV made by electrospinning PCUU mounted on a biodegradable Mg alloy stent is implantable and shows good valvular function during the acute phase in the pulmonary position. Additional study will be required to modify the biodegradable Mg stent to be less thrombogenic and have slower degradation characteristics.

Developing urinary catheter safety devices and improving tissue engineered urethral scaffolds through 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 trialed in 100 patients with the valve activating 7 times and requiring subsequent manipulation to allow for full inflation. The mechanical characterisation data generated in this study 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 the native tissue than current gold standard tissue engineered constructs. Subcutaneous murine implants are planned to determine the regenerative potential of this mimetic tissue engineered scaffold material.

A single localized dose of ultrasound-responsive release hydrogel improves long-term survival of a vascularized composite allograft

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Purpose
More than 185,000 amputations occur in United States each year. The most common is a partial hand amputation (61,000). Vascularized composite allotransplantation (VCA), in the form of hand transplantation, provides another option for hand reconstruction. The skin component of VCA is highly antigenic and mandates high doses of systemic immunosuppression. Oral dosing of these drugs leads to fluctuating blood levels, risking toxicity or lack of efficacy. We propose drug delivery system that can provide sustained or on-cue triggered drug release up on ultrasound (US) stimulation in graft tissues, minimizing overall drug exposure and facilitating long-term VCA survival with no systemic complications.

Method
An injectable, re-loadable hydrogel was prepared. We evaluated the feasibility and efficacy of the alginate gels in absence and presence of US. Brown Norway to Lewis hind limb transplanted rats (6/group) received TAC 1mg/kg/day intraperitoneally (Group 1), or a single dose of gel loaded with either TAC 10mg (Group 2), or TAC+ Rapamycin 10 mg each (Group 3). TAC levels were measured using LC-MS/MS. In addition to allograft survival, systemic toxicity was evaluated using percent change in body weight (BW), and creatinine clearance (CrCL).

Results
TAC and Rapa exhibited a low baseline level (without fluctuation) of release from alginate gels in the absence of ultrasound while pulsatile ultrasound application triggered drug release, leading to increased drug levels after each pulse. Animals in group 3 received a single localized dose of alginate gel (TAC and Rapa) sustained their allograft survival for >100 days, and maintained high drug levels locally at the gel injected limbs, when compared to the levels in the other contralateral limb (**$P<0.01$). No significant change in BW and CrCL was observed in animals received a single localized dose of alginate gel as compared to animals in group 1 received standard systemic therapy (*$P<0.05$).

Conclusion
Smart hydrogels with desired baseline and on-demand release of drugs for the in vivo studies was prepared. TREAT system provides an optimal, sustained drug release in graft tissues with stable, low blood levels facilitating long-term VCA survival/outcomes with no systemic adverse effects. We developed dibenzocyclooctyne (DBCO)-immunosuppressant prodrugs that can be reloaded into the azido-modified alginate gels via Click chemistry, with the goal of refilling the alginate scaffolds with immunosuppressive drugs once the encapsulated drugs are running out and thus maintaining the immunosuppressive effect for a long term without replacing the gel scaffolds via surgery method.

Enzyme Responsive-Release Hydrogel for Targeted Immunosuppression in Vascularized Composite Allotransplantation

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Purpose
More than 30,000 people receive organ transplants every year in the US. Vascularized composite allotransplantation (VCA) is the newest realm of solid organ transplantation. The skin component of VCA is highly antigenic and mandates high doses of systemic immunosuppressive drugs. Oral dosing of these drugs leads to fluctuating blood levels risking toxicity or lack of efficacy. We propose a drug delivery platform that can not only provide sustained drug release but also on-cue triggered drug release in response to MMP-2 and MMP-9 that are significantly upregulated during rejection in graft tissues, minimizing overall drug exposure and facilitating long-term VCA survival with no systemic complications.

Method
An injectable, self-assembled, and biocompatible drug eluting hydrogel was prepared. We evaluated feasibility and efficacy of the system in vitro and in vivo. Allogeneic transplanted rats (6/group) received single dose of GEL IT hydrogel loaded with either no drug (control, group 1), Tacrolimus (TAC) 7mg (group 2), or TAC 3.5mg and Rapamycin (Rapa) 3mg (combined), subcutaneously injected into the transplanted hind limb (group 3) or contralateral un transplanted limb (group 4). Drugs levels were measured using LC-MS/MS. Allograft survival and systemic toxicity were evaluated.

Results
TGMS hydrogels released the drugs predominantly in response to proteolytic enzymes. A one-time local injection of TAC (group 2) or TAC and Rapa (group 3) laden hydrogel that injected in the transplanted hind limb prolonged the allograft survival for >100 days. Drugs release from TGMS gel and blood levels were proportional to the degree of immune activation, and maintained within the therapeutic range 5-15 ng/ml without fluctuations. No significant change in body weight or kidney function was observed in group 3, as compared to other groups ($P<0.05$). Expression of regulatory marker, FOXP3 indicates peripheral tolerance.

Conclusion
We successfully developed, for the first time, a smart hydrogel drug delivery system with sustained baseline and on-cue triggered drug release in response to protolytic enzymes that are significantly up-regulated during rejection in graft tissues, minimizing overall drug exposure and facilitating long-term VCA survival with no systemic complications.

Allograft Targeted Delivery of Tacrolimus prolongs Vascularized Composite Allograft Survival with Negligible Blood Levels

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Purpose
Oral dosing of tacrolimus (TAC) leads to fluctuating blood levels of the medication, risking toxicity or lack of efficacy and rejection. We propose a drug delivery platform that consists of an encapsulated sustained-release version of oral TAC that provides sustained drug release into the graft tissues and regional lymph nodes, while minimizing systemic blood levels. This facilitates VCA survival without the need for daily intake of oral TAC with its complications.
Methods
TAC loaded polycaprolactone discs were prepared by solvent casting. Following orthotopic hind limb allotransplantation, animals (n=6/group) received no treatment (Group 1), TAC 1mg/kg/day intraperitoneally (Group 2), one disc in the un-transplanted limb (Group 3), or in the transplanted limb (Group 4). TAC levels were measured using LC-MS/MS. Allograft survival and systemic toxicity were evaluated.
Results
A single TAC disc resulted in blood levels between 2 to 5 ng/ml for nearly 50 days. High levels of TAC were achieved locally when the disc was implanted into the transplanted limb, when compared to levels in the contralateral limb (**$P<0.01$). These levels could inhibit immune activation and sustained the allografts for >150 day (Group 4), while animals in group 3 had median survival 71 ± 7 days (* $p=0.02$). No significant change in BW, glucose levels, and CrCL rates was observed in group 4, as compared to Group 2 (*$P<0.05$).
Conclusion
A single TAC disc implanted into the transplanted limb was effective in sustaining allograft survival via loco-regional immunosuppression (IS), without systemic side effects. Our study offers an alternative to the current treatment paradigms which use systemic IS, to loco-regional IS using locally implantable biomaterials.

Retrograde Hemorrhage and Ischemic Injury after REBOA in a Porcine Model of Uncontrolled Aortic Injury

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Introduction: Resuscitative Balloon Occlusion of the Aorta (REBOA) has gained popularity as a less invasive approach to temporize traumatic noncompressible hemorrhage, yet mortality remains over 70%. Although associated injuries may account for some deaths, contributions from ischemia and ongoing retrograde bleeding are also likely, with REBOA occlusion frequently in Zone 1 (descending thoracic aorta) and often greater than 20 minutes. This study examined retrograde blood loss and ischemic injury after REBOA in a porcine model of aortic injury.

Methods: Six anesthetized swine with invasive hemodynamic and neurophysiologic monitoring (Motor Evoked Potentials and Somatosensory Evoked Potentials) underwent 8 Fr femoral access and Zone 1 positioning of a REBOA balloon prior to aortic injury. The thoracic aorta was injured with a 22 Fr dilator, followed by aortography and immediate REBOA inflation proximal to the injury. Profound deterioration of the first three animals with one hour of REBOA prompted the next three animals to undergo only 30 minutes of REBOA. Blood loss was recovered with a cellsaver. Animals underwent permanent stent repair of the aortic injury and resuscitation with the intent to recover.

Results: Despite proximal hemorrhage control documented angiographically, blood loss from retrograde bleeding was substantial averaging 3.7 L and 3.5 L for the 30- and 60-minute groups, respectively. After balloon inflation, mean pressure fell an average of 62 mmHg within 20 minutes ($p < 0.001$), while cardiac output decreased 20-40%. In the lower extremities, neuromonitoring revealed ischemic loss of motor signals at a mean of 27 minutes. Even after resuscitation with blood, bicarbonate, saline, and pressors, all six animals arrested shortly after balloon deflation, amidst falling bicarbonate ($p<0.001$) and rising lactate ($p>0.01$) relative to baseline.

Conclusions: Retrograde hemorrhage is an underappreciated event during REBOA control of aortic injuries, which appears to contribute to spinal cord ischemia, tissue ischemia, and death. This study suggests that improved outcomes for noncompressible hemorrhage will require balance of competing goals of hemorrhage control and distal perfusion.

Muscle-powered Counterpulsation Ventricular Assist Device (mVAD) for Long-term Cardiac Support

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Conventional long-term ventricular assist devices (VADs) continue to be extremely problematic due to infections caused by percutaneous drivelines and thrombotic events associated with the use of blood-contacting surfaces. Here we describe a muscle-powered ventricular assist device (mVAD) that avoids both these problems by using an internal muscle energy converter (MEC) to drive a non-blood-contacting counterpulsation balloon. The MEC was developed previously in this lab and operates by converting the contractile energy of the latissimus dorsi muscle (LDM) into hydraulic power that can be used, in principle, to drive any blood pump amenable to pulsatile actuation. The two main advantages of this implantable power source are that it 1) significantly reduces infection risk by avoiding the need to transmit energy across the skin, and 2) improves patient quality-of-life by eliminating all external hardware components. Extra-aortic balloon pumps (EABPs), which compress the external surface of the ascending aorta during the diastolic phase of the cardiac cycle, offer another critical advantage in the setting of long-term circulatory support in that they increase cardiac output and improve coronary perfusion without touching the blood. The goal of this work is to combine these two technologies into a single circulatory support system that eliminates driveline complications and avoids surface-mediated thromboembolic events, and thereby providing a safe, tether-free means to support the failing heart over extended – or even indefinite – periods of time.

A Clinically Relevant Rabbit Surgical Model of Pelvic Reconstruction to Evaluate the Regenerative Immune Response to Novel Surgical Materials

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Pelvic organ prolapse, 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, the number of complications such as chronic pain and mesh erosion/exposure in women with vaginal mesh implants were reported at rates as high as 10-20 %. This indicates a limited understanding of the host response to mesh in vaginal tissue and strategies to reduce these complications.

Utilizing a novel surgical technique in New Zealand white rabbits, we implant mesh using the "gold standard" abdominal sacrocolpopexy procedure and evaluate changes in the immunologic response at early (14 days) and tissue remodeling outcomes at late stages (90 and 180 days) of implantation. The procedure begins with an initial hysterectomy followed by securing two 3 x 12 cm2 pieces of mesh along both sides of the vaginal wall. The remaining flaps at the top are then secured to a ligament in the sacral/lumbar space, creating the support to the pelvic organs. Upon closing the incision, mesh is implanted in the abdominal muscle. Both of these implantations of mesh allow for the assessment of the immune response in the pelvic area (relevant for prolapse patients) and in the abdominal area (relevant for translation from hernia repair). The mesh-tissue complex was removed from each rabbit and processed for histological staining as well as immunolabeling of immune cells, such as macrophages. Extracellular matrix protease assays and mechanical integrity of the tissue also evaluate the overall inflammatory response associated with each implant.

An ideal mesh would provide mechanical support to the pelvic floor while decreasing the inflammatory response and increasing integration with the surrounding native tissue. The results of this study show that implants into vaginal tissues elicited an increased host inflammatory response at 14 days as compared to those in the abdominal wall. However, at chronic time points the inflammatory response in the vagina was reduced as compared to that in the abdominal cavity.

In vivo imaging of reactive astrocyte following chronic implantation of microelectrode array

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Implantable silicon microelectrode arrays can record high-density and high-spatial resolution signal from neurons across the brain. These microarrays are highly scalable and has potential to revolutionize neuro-prosthetic devices; however, microelectrodes are limited by their diminishing ability to consistently record high-quality signals over time, due to chronic tissue response1. The tissue encapsulation that forms chronically consists hypertrophic astrocytes, fibroblasts, and meningeal cells that surrounds an activated macrophage and microglial1. In healthy brain astrocytes maintain blood brain barrier (BBB), provide nutrients to the neuron and maintain extracellular ion homeostatic, but following injury it plays significant role in repair of damaged tissue and scar formation2. The BBB leakage, mechanical strain and injury caused during microelectrode insertion immediately activates the surrounding microglia3, and subsequently activates surrounding astrocytes, but to reach peak activation of astrocyte can take up to one to two weeks4. Even though activation of microglia following microelectrode insertion has been characterized using in vivo imaging3; astrocyte behavior has largely been inferred from postmortem analysis1. In vivo characterization of astrocyte activation following microelectrode insertion will help developing intervention strategy to reduce chronic tissue response and improve longevity of microelectrode recordings. To examine the mechanisms that recruit astrocytes to the site of insertion injury, we used in vivo two-photon laser-scanning microscopy to follow the response of GFP-labeled astrocytes in FVB/N-Tg(GFAPGFP) 14Mes/J transgenic mouse visual cortex over weeks after acute injury. Live imaging revealed a marked heterogeneity in the reaction, with one subset retaining their initial morphology, and another directing their processes toward the lesion and encapsulating the probe. The activated astrocytes remained hypertrophic following weeks after insertion. Juxtavascular astrocytes were more prone to activation than the ones close to probe but distant from vasculature.

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Damage Control of Caval Injuries in a Porcine Model using a Retrievable RESCUE Stent

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Objective: Early hemorrhage control is essential to reduce the significant mortality associated with traumatic injuries of the vena cava. Conventional approaches of compression, open repair, or permanent stenting each present logistical challenges on the battlefield or in the trauma bay. A retrievable stent graft would allow for rapid hemorrhage control in the pre-operative setting. This study details a refined retrievable RESCUE stent for percutaneous delivery and was examined in a porcine survival model of penetrating caval hemorrhage.

Methods: A retrievable caval stent of 23 mm diameter was constructed with a “petal and stem” design using nitinol wire followed by covering with polytetrafluoroethylene. Seven Yorkshire pigs (79-86 kg) underwent 22 Fr injury of the infrarenal vena cava with intentional class II hemorrhage (1200 mL). Percutaneous deployment of the RESCUE stent was used to temporize hemorrhage for 60 minutes, followed by resuscitation with cellsaver blood and permanent caval repair. Hemorrhage control was documented with photography and angiography. Vitals were recorded and labs were measured out to 48 hours postoperatively.

Results: The 9 Fr profile of the caval RESCUE stent allowed for both rapid deployment and recapture. Following intentional hemorrhage after caval injury, animals revealed a significant drop in mean arterial pressure (average 30 mm Hg), acidosis, and elevated lactate compared to pre-injury. Uncontrolled hemorrhage resulted in death in under 9 minutes. The RESCUE stent achieved hemorrhage control in under one minute after venous access in all animals. All animals were successfully recovered after permanent repair, although one animal was removed from the study at 24 hours postoperatively for a recurrent tachyarrhythmia. Both pH and lactate levels normalized by 24 hours.

Conclusions: A retrievable RESCUE stent achieved rapid percutaneous hemorrhage control after a significant traumatic injury of the vena cava, and allowed for successful recovery of all injured animals. Further development of this approach may have utility in pre-operative damage control of caval injuries.

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.

Non-neoplastic extracellular matrix components abrogate primary human glioma cell malignancy

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Gliomas are the most lethal and common primary tumor type in the central nervous system (CNS) across all age groups; affected adults have a life expectancy of just 14 months. As glioma cells invade the surrounding normal parenchyma they remodel the composition and ultrastructure of the surrounding extracellular matrix (ECM), suggesting that the native normal microenvironment is not ideal for their survival and proliferation. Recent reports describe suppressive and/or lethal effects of mammalian ECM hydrogels derived from normal (non-neoplastic) sources upon various cancer types.

We observed that ECM hydrogels derived from porcine dermis, small intestine, or urinary bladder all decreased the viability of primary glioma cells in vitro, with urinary bladder extracellular matrix (UBM) having the most dramatic effects. We subsequently determined that the saline-soluble fraction of UBM (UBM-SSF), devoid of the fibrillar, macromolecular components of ECM, is sufficient to recapitulate this detrimental effect upon neoplastic cells in vitro. In a cell viability assay (MTT) normalized to the media treatment, non-neoplastic CHME5 and human foreskin fibroblast cells scored 103% and 114% after 48 hours when treated with UBM-SSF. Two primary high grade glioma cell types scored 17% and 30.5% with UBM-SSF (average of N=2). Time-lapse video showed CHME5 cells thriving in the presence of UBM-SSF while most glioma cells shriveled and died. Videos with Nucview dye, fluorescent upon cleavage by active caspase-3, confirmed that glioma cells underwent apoptosis while CHME5 cells did not. In preliminary animal experiments, large primary glioma tumors in the flanks of athymic nude mice were resected and replaced with either UBM saline-soluble fraction or Matrigel (an ECM product of neoplastic cell origin). After 7 days the resection sites with UBM saline-soluble fraction had very little tumor regrowth compared to the dramatic recurrence seen in the Matrigel injection sites (N=2).

These findings indicate that non-neoplastic ECM contains potent homeostatic regulators capable of abrogating malignancy. Delivering soluble fractions of ECM to a tumor site may represent a novel approach to cancer therapy, sidestepping traditional cytotoxic therapies in favor of utilizing putative endogenous anti-tumor pathways.

In Vitro Characterization of a Modular Pump Lung Capable of Multiple Respiratory Assist Applications

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The Modular Extracorporeal Lung Assist System (ModELAS) is a wearable pump lung that utilizes a modular design to enable multiple types of respiratory support. The ModELAS pump can be configured with hollow fiber membrane bundles of varying surface areas to accommodate adult or pediatric patients as well as full respiratory support or low flow CO2 removal. The resulting versatility increases the patient population to benefit from the ModELAS by an order of magnitude relative to typical systems not meant for low-flow CO2 removal. This study used CFD and in vitro methods to evaluate ModELAS performance in a variety of respiratory assist applications.

The ModELAS configured with a 0.3 m2 bundle (ModELAS-0.3) was evaluated for pediatric support at flows of 1-2.5 L/min. Configuration with a 0.65 m2 bundle (ModELAS-0.65) was evaluated for adult support at 1-3.5 L/min and low-flow CO2 removal at 250-750 mL/min. Pump curves were obtained for each bundle over the relevant flow rates using a blood analog. Gas transfer rates were evaluated in bovine blood for each bundle and flow rate range. The normalized index of hemolysis (NIH) and therapeutic index of hemolysis (TIH) were measured in bovine blood for the ModELAS-0.3 at 2.5 L/min and ModELAS-0.65 at 500 mL/min, respectively.

CFD results showed minimal flow stasis in the blood flow path and uniform intra-bundle flow for all conditions. The ModELAS generated targeted blood flow rates in each setting against the resistance associated with the corresponding intended cannulas. The ModELAS-0.3 and ModELAS-0.65 achieved O2 transfer rates of 105 and 207 mL/min, respectively, at the maximum intended flow rates. The CO2 removal rate of the ModELAS-0.65 was 83 mL/min at a blood flow rate of 500 mL/min. Hemolysis was low with an NIH of 0.029 g/100 L and TIH of 0.142 g/100 min for the ModELAS -0.3 and ModELAS-0.65, respectively. Thus, the ModELAS meets the targeted pumping and gas transfer specifications for the described applications and exhibits low blood damage.

Successful 30-day Sheep Studies of a Wearable Pumping Artificial Lung

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The Paracorporeal Ambulatory Assist Lung (PAAL) is an oxygenator and blood pump integrated into a compact unit that is wearable and enables patient mobility. The PAAL is meant to provide long-term respiratory support as a bridge to transplant or recovery. This work aims to evaluate the extended in vivo performance of the PAAL in a 30-day sheep study.

The PAAL was connected to healthy adult sheep (45-65 kg) via cannulation of the right external jugular with a dual-lumen cannula. PAAL pump speed was set at the highest setting possible without frequent suction and kept constant throughout the study. Following surgery, sheep were recovered and housed in a fixed tether pen while wearing the PAAL in a holster. Anticoagulation was maintained via a continuous heparin infusion. PAAL blood flow rate was measured continuously while device blood gases were taken at least twice per week. Blood cell counts, chemistry, plasma free hemoglobin (PfHb), and platelet/leukocyte activation were measured weekly.

Two of three animals survived the entire study duration without device exchange. PAAL blood flow rates were variable early in the study before stabilizing at 1.3-2.1 L/min over the final two weeks. Blood at the PAAL outlet was always fully saturated and trends in oxygenation were primarily determined by blood flow rate. PfHb did not substantially increase relative to baseline values with the exception of an increase following a blood transfusion. Platelet and leukocyte activation remained less than 20% for all animals. Explanted devices exhibited minimal thrombus deposition on the fiber bundle and more significant thrombus underneath the impeller. Complications were acceptable and included the following: a hematoma in one animal lead to anemia and required a blood transfusion and the third study was prematurely terminated on postoperative day 8 due to a fractured cannula (corresponding data excluded from primary analysis). This study is ongoing, but results thus far indicate positive PAAL performance in an extended use setting.

Advancing a System for Generating Universal Blood Plasma

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Traumatic injury represents one of the most common reasons for mortality and accounts for 14.2% of all fatalities. A study of US military death distribution indicates 91.5% of potentially survivable deaths in warzones were due to hemorrhagic shock. Clinical management of trauma, shock, burn injury, and surgeries often require massive plasma transfusions. Fresh, frozen plasma represents 29% of the blood products in transfusions according to the armed forces. This projects aims to address the currently unmet need for a device capable of generating ‘universal blood plasma’. We utilize affinity column chromatography to specifically extract type A and type B antibodies from the blood plasma. The bead type being used is Sepharose CL-6B – a crosslinked agarose bead with hydroxyl groups covering its surface. Materials specific to binding antibody types A and B are attached to a high molecular weight polymer (~1000kDa) and linked to the surface of the affinity bead by way of an activated support. The activated support material used in early work was cyanogen bromide (CNBr). CNBr is an effective molecule at binding itself to the affinity bead surface and reacting with the polymer chain containing type A and B antigens. However, there are multiple drawbacks to this material. To start, CNBr is a highly toxic material. Its byproduct is hydrogen cyanide which can be fatal in very small atmospheric concentrations. Secondly, the attachment of CNBr to the surface of the agarose bead creates an isoureic bond. The isoureic bond will lead to nonspecific interactions in the system and also will be unstable in aqueous conditions, leading to ligand leakage. Carbonyl Diimidazole (CDI), a safer material, is being tested to replace CNBr. Preliminary results show its similar ability to attach to the surface of the agarose bead. Future work aims to show its improved stability over time versus CNBr.

RegenMatrix - A Growth Factor-free Bone Graft for Craniofacial Bone Regeneration

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Here, we report development and efficacy of patent-pending bioresorbable “RegenMatrix” as a platform technology to fulfill the current unmet clinical need of low cost, growth factor-free osteoinductive graft materials for craniomaxillofacial bone regeneration. RegenMatrix is a hierarchical bioresorbable biomaterial self-assembled from oppositely charged polysaccharides to form aligned fibrous structure mimicking collagen, extracellular matrix of the native bone. We engineered two types of collagen-inspired ‘RegenMatrix’ (chitosan-gellan gum (CGG) and chitosan-kappa-carrageenan (CKC)). The structural mimicry of collagen is translated into the biomimetic mineralization in vitro, where growth of amorphous calcium phosphate and crystalline apatite-like minerals is observed inside and on the surface of RegenMatrix, respectively. In a critical size calvarial defect model in mice, non-mineralized CKC processed as films showed significantly higher bone volume as compared to empty defect in micro-computed tomography (μCT) quantification, however, the defect was still far from closure. More interestingly, pre-mineralized lyophilized CKC RegenMatrix films further enhanced bone regeneration significantly without added growth factors compared to empty defect and non-mineralized RegenMatrix. In a recently completed critical size ulna defect study in rabbits, RegenMatrix processed as cylindrical scaffolds showed onset of regeneration as early as 4 weeks and enhanced regeneration at the end of 12 weeks compared to the empty defect group. These in vivo studies suggest that RegenMatrix is a flexible platform technology and can be processed in any shape and sizes; pre-fabricated in the lyophilized, hydrated or even injectable form. RegenMatrix can conform to and maintain the shape of the defect. In conclusion, promising efficacy results in both mice and rabbit models suggest that RegenMatrix has great potential to overcome disadvantages of current autografts (morbidity) and collagen with BMP2 (bone overgrowth, cost, poor control over bone regeneration, contra-indicated in cancer patients) and alloplasts (e.g. hydroxyapatite, b-tricalcium phosphate etc. that show unpredictable osteoconductivity and resorbability). These advantages and broader applicability of RegenMatrix make it ideal for tapping into the vast clinical need of flexible bone graft materials in the oral and maxillofacial applications.

Diffuse optical imaging for breast cancer monitoring

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When breast lesions display "probably benign" features on x-ray mammography, a decision must be made between immediate biopsy and repeat imaging at six-month intervals. A device capable of frequent monitoring, possibly in the patient's home, could provide earlier detection of malignancy while reducing potentially unnecessary biopsies. There is also a need for frequent monitoring during neoadjuvant chemotherapy for prediction of treatment outcome. Breast tumors are known to be highly vascularized, resulting in a greater hemoglobin concentration. Such changes with respect to healthy tissue can be measured with diffuse optical spectroscopic imaging techniques. In addition to vascular changes, breast tumors also tend to be stiffer than surrounding tissue. We have developed an imaging device which takes advantage of both mechanical and optical contrast in the breast.

The device is based on optical imaging using spatially modulated light (spatial frequency domain imaging (SFDI)) and operates in reflectance mode. Tissue compression is used to enhance contrast of the lesion in respect to the background by effectively decreasing the distance from a stiff lesion to the tissue surface. A single-wavelength SFDI system has been tested on flexible phantoms, each containing one stiff inclusion, with optical properties of background and inclusion being representative of healthy breast tissue and breast tumor, respectively. Inclusions were detectable up to a depth of 13 mm, with contrast increasing with phantom compression. A multi-wavelength prototype has been developed which is capable of monitoring tissue oxygenation of hemoglobin in addition to hemoglobin concentration. Data from phantom experiments will be presented.

Two-Photon Imaging Reveals Processes Extension and Cell Body Migration of Reactive NG2 Glia During Brain Injury

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Neural interface technology provides direct sampling and analysis of electrical and chemical events in the brain in order to better understand neuronal function and treat neurodegenerative disease. However, intracortical electrodes experience inflammatory reactions that reduce long-term stability and functionality and are understood to be facilitated by activated microglia and astrocytes. Emerging studies have identified another cell type that participates in the formation of a high-impedance glial scar following brain injury; the oligodendrocyte precursor cell (OPC). These cells maintain functional synapses with neurons and are a crucial source of neurotrophic support. Following injury, OPCs migrate toward areas of tissue injury over the course of days, similar to activated microglia. The delayed time course implicates these OPCs as key components in the formation of the outer layers of the glial scar around the implant. In vivo two-photon laser scanning microscopy (TPLSM) was employed to observe fluorescently-labeled OPC and microglia reactivity up to 72 hours following probe insertion. OPCs initiated extension of cellular processes (2.5 ± 0.4 μm h-1) and cell body migration (1.6 ± 0.3 μm hour-1) toward the probe beginning 12 hours after insertion. By 72 hours, OPCs became activated at a radius of about 190.3 μm away from the probe surface. This study characterized the early spatiotemporal dynamics of OPCs involved in the inflammatory response induced by microelectrode insertion. OPCs are key mediators of tissue health and are understood to have multiple fate potentials. Detailed spatiotemporal characterization of glial behavior under pathological conditions may allow identification of alternative intervention targets for mitigating the formation of a glial scar and subsequent neurodegeneration that debilitates chronic neural interfaces.

Evaluation of Device-based Mechanical Interlocking System for Sustained Oral Delivery to the Gut

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Oral drug delivery is the preferred delivery system for many drugs, however it places the burden of adhering to a drug regimen on the patient. An orally delivered device that can administer doses for weeks, during its dissolution, can improve patient compliance and consequently patient outcomes. Current extended release systems are limited to about two days, or the average transit time through the GI tract. This only provides up to two days of dosing from a typical extended release system. Mucoadhesion, or adhesion to the mucus membranes in the GI tract, would enable a drug delivery system to release drugs over weeks and would not rely on GI transit time.

Previous studies are focused on adhering drug delivery systems to the mucus layer of the mucosal membrane. This can be improved upon because mucus in the GI tract has a short lifetime. It is constantly in the process of being destroyed and recreated. In our work, we explore a mechanical interlocking adhesive that forms a bond to the intestinal epithelial layer beneath mucus. A device adhered to the epithelium will be able to persist in the GI tract for extended periods of time, through the benefits of bonding to solid tissue.

The adhesive we tested consists of a micropatterned elastomer with geometry that mechanically interlocks with the villi in the intestine. Friction, the mechanical properties of the adhesive/villi interface, and the Van der Waals forces between the two materials create the adhesive bond. Finite Element Modelling (FEM) and experimental force measurement was used to determine the relative strength of this system. Our tests indicated that a significant adhesive bond could be formed with this approach. Further research could lead to a new class of mucoadhesive materials.

Magnetic field assisted localization of ADMSCs with iron nanoparticles

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Background: Periadventitial delivery of adipose-derived mesenchymal stem cell treatment has demonstrated that abdominal aortic aneurysm progression in a murine elastase-induced model was halted1. The localized delivery method required the mice to receive ADMSCs through a subcutaneous port leading to a sponge implanted on the anterior portion of the aorta. In order to test this potential therapy in a large animal model and to promote clinical translation, a minimally-invasive and robust means of localized delivery of the stem cells is needed.

Objective: Determine the influence of a neodymium permanent magnet (surface field of ~4100 Gauss) on ADMSCs loaded with iron nanoparticles.

Methods: Adipose derived mesenchymal stem cells (ADMSCs) were cultured at 37o C with adipose-derived stem cell growth medium (Cyagen Biosciences, Inc., CA. USA) with media changes every 2-3 days. Before removing the ADMSCs from their flasks, 200 nanometer iron nanoparticles were included in the final media change and incubated for 24 hours. A custom designed and 3D printed mixing apparatus was used to inject the fibrinogen (3.7 mg/ml), thrombin (0.21 units/ml) and nanoparticle-loaded ADMSCs (6 ml, 5x105 cells/ml) onto the anterior abdominal aorta of a sacrificed rat that was placed on a stage with (n = 3) and without (n = 3) a neodymium permanent magnet. The fibrin gel was allowed to set for 30 minutes at 37o C with the magnet still placed. A large section of the rat was excised and fixed in paraformaldehyde (PFA) overnight and sectioned.

Results: The experimental group (ADMSCs, iron nanoparticles, fibrin and magnet) demonstrated that the cells were localized onto the anterior surface of the aorta while being encapsulated by fibrin gel. The control group (ADMSCs, nanoparticles, fibrin without magnet) revealed a homogeneous distribution of cells within the fibrin gel.

Discussion: Localization of nanoparticle-loaded ADMSCs with a strong permanent magnet was demonstrated to be a potential method for cell delivery. Future in vivo studies will be performed to ensure the efficacy of this delivery mechanism.

Adipose tissue engineered model for studying metabolic diseases

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Over 35% of U.S. adults are classified as obese. In obese states, energy intake exceeds the storage capacity of adipose tissues triggering an inflammatory response. While it is hypothesized that a link between these changes and the onset of type-II diabetes exists, the mechanism remains unknown. Current progress in determining the mechanistic link is hindered by the lack of physiologically-relevant human adipose tissue models. Human adipose microenvironments were developed and tested for long term responsiveness to stimuli hypothesized to alter disease mechanisms (inflammatory mediators), metabolic behavior, and therapeutic potential. By integrating multiple cell types, including human primary mature adipocytes and stromal vascular cells (endothelial cells, pericytes and preadipocytes) in a 3D silk matrix, we have developed a comprehensive in vitro adipose tissue model. On going work is aimed at recapitulating disease processes in this system. Ultimately we would like to pair these adipose tissue models with in silico multivariate statistical modelling techniques to identify key markers of disease progression. Together in vitro and in silico models will be used to characterize altered cellular components of the adipose tissue in the overfed state, during the development of type-II diabetes, and in response to metabolic stressors. Statistical modeling will track the variation between different human patient samples and therefore will provide a method of determining whether certain populations uniquely react to different stimuli. These insights will be used to inform recommendations for preventative interventions and to discover new treatments for type-II diabetes.

Scaffold-free Dental Pulp Cell Sheets to Enhance Peripheral Nerve Regeneration

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Introduction:
Peripheral nerve damage is a commonly encountered clinical problem caused by trauma, disease, or surgical injury. The current gold standard treatment utilizes autologous nerve grafts; however, this requires a prolonged repair time and full functional recovery is not achieved. Neurotrophic factors (NTF) are proteins known to enhance axon regeneration and growth. Dental pulp tissue contains a population of stem/progenitor cells (DPC) that secrete NTFs a characteristic likely due to their neural crest origin. Furthermore, these cells are easily accessible from autologous sources. The goal of this study was to develop and characterize scaffold-free DPC sheets as a NTF delivery system. We hypothesize that DPC sheets will express NTFs including brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factors (GDNF) and neurotrophin-3 (NT-3), and will accelerate repair of damaged nerves and improve functional recovery.

Materials & Methods:
In this study, we fabricated scaffold-free cell sheets by culturing DPCs to super confluence with and without fibroblast growth factor 2 (FGF2). NTF gene expression of DPC sheets was assessed using qRTPCR. DPC sheets secretome was used to culture SHSY-5Y neurons to test its effect on neurite extension in vitro.

Results:
DPC sheets were formed that are robust and can be easily handled. DPC sheets expressed high level of BDNF, GDNF, NT3 genes and this effect was enhanced by the addition of FGF2. DPC sheet secretome enhanced neurite extension in SHSY-5Y neurons indicating that DPC sheets have a positive functional effect on neurons.

Conclusion:
DPC sheets can be formed which secrete neurotrophic factors and enhance neurite extension in neurons. Scaffold-free DPC sheets show great promise as a new therapy to accelerate the regeneration of damaged peripheral nerves and improve functional recovery.

Tailoring TGFB2 release from electrospun scaffolds for vascular tissue engineering

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Tissue engineering has gained attention for the development of small diameter tissue engineered vascular grafts (TEVGs) to treat coronary heart disease. A TEVG has to support the growth and induce matrix deposition of smooth muscle cells (SMCs) as an analogy for the media layer of a coronary artery. To do so, the TEVG scaffold can possess additional biofunctionality acting as a releasing matrix of signaling factors such as TGFB2 to control SMCs phenotype. We have previously demonstrated that exogenous TGFB2 differentially modulates SMCs proliferation and collagen deposition in electrospun scaffolds. Therefore, the goal of this work is to build a TEVG that allows for the control release of TGFB2 using the degradation times of electrospun natural and synthetic materials such as gelatin, tropoelastin and PCL. Towards this end, polymeric solutions with different ratios of biodegradable gelatin/PCL and tropoelastin/PCL were electrospun to fabricate scaffolds loaded with TGFB2. Scaffold morphology, porosity, release kinetics and bioactivity of the eluted TGFB2 were assessed. Scaffolds composed by tropoelastin/PCL have a TGFB2 burst release in the early time points. The gelatin/PCL construct had a slow continuous elution of TGFB2 over the course of two weeks. The scaffolds with majority protein in the protein/PCL blend have a greater percentage of release than the ones with majority PCL. The released TGFB2 remained bioactive over two weeks as assessed by the SMCs proliferation assay. The use of synthetic and natural polymers in a blend will allow us to control the release of TGFB2 by taking advantage of their degradation times. In this way we can systematically modulate SMCs phenotype in the electrospun scaffold for cardiovascular tissue engineering applications.

Using Sub-Nuclear Sensors to Determine Mechanical Features of Epithelial Monolayers

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Intracellular force generation is critical to a cell's function particularly in areas such as wound healing, and embryonic development. We have developed a technique, which uses the nucleus as a force output within cells to map force landscapes within cell monolayers and address fundamental questions in the field of biomechanics such as "How far can a cell feel?". This technique is termed Sensors from IntraNuclear Kinetics (SINK), in which fluorescent, chromatin bound, proteins within the nucleus are tracked over time and mean square displacement of these particles is calculated. Upon fitting these displacements to a power law, we show that the exponent serves as a relative measure of intracellular force propagation. We demonstrate this by decreasing cellular force generation, using a ROCK inhibitor, and by physical decoupling of the nucleus from the cytoskeleton through disruption of the linker of the nucleus and cytoskeleton complex. In both cases we see a reduction in intranuclear motion, compared to control cells. Next, we investigate the changes in chromatin dynamics as cells transition from subconfluence, to confluent monolayers. These changes are likely due to changes in cytoskeletal structure which impacts force propagation. Then, we use our technique to mimic tissue fibrosis by investigating how substrate stiffness impacts force propagation through monolayers. Finally, we investigate the role that cellular point defect, in an otherwise homogeneous monolayer, has on both nearby and distant cells, to address how far a cell can "feel" laterally. This work demonstrates the power of the SINK technique to address fundamental cellular mechanics questions in disease models and experimental set ups.

Local Administration of FK506 with Impregnated Nerve Wraps Accelerates Nerve Regeneration

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Introduction
Peripheral nerve injuries can lead to permanent functional disabilities. Systemic FK506 administration can improve recovery and 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 while avoiding off-target side-effects. This study investigates the utility of a novel FK506-infused nerve wrap in treating peripheral nerve injuries in a rat infraorbital nerve transection and repair model.

Methods
Infraorbital nerve transection surgeries were performed on two groups (n=5) of Lewis rats. Transected nerves were repaired primarily with (treatment group) or without (no treatment group) the addition of a Poly(ester urethane) urea (PEUU) wrap infused with 20mg of FK506. Trigeminal ganglion cell recordings, objective sensory testing, directional sensitivity, maximal response, and receptor compositions were analyzed at four and six weeks postoperatively. Blood and tissue samples were analyzed for FK506 concentration using LCMS spectrometry.

Results
Treatment group rats were found to have increased response magnitude at 4 weeks postoperatively in comparison to no treatment group ($p<.013$). FK506 blood levels at 4 and 6 weeks were close to the limit of quantification (<2ng/ml), whereas concentration within the tissues of interest were much higher.

Conclusion
This study investigates the use of a FK506-impregnated nerve wrap to improve functional recovery following peripheral nerve injury. The FK506 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.

Scaffold-free Tissue Engineering for Periodontal Regeneration

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Introduction: The periodontium is a multi-tissue structure that functions to anchor teeth to the jaw and contains the mineralized tissues cementum and alveolar bone that are bound together by the periodontal ligament (PDL). A major challenge in periodontal regeneration is recreating this complex structure. Previously we have shown the scaffold-free tissue engineering facilitates cells to self-assemble into multi-tissue constructs. The periodontal ligament contains a population of stem/progenitor cells (PDLCs) that can differentiate into cells of the cementum, periodontal ligament and alveolar bone. The goal of this study is to assess if scaffold-free tissues engineered from PDLCs can self-assemble into the complex structure of the periodontium.

Methods: Scaffold-free constructs were engineered using human PDLCs isolated from 3rd molars. PDLCs were cultured in osteogenic media in wells of 6-well plates. Upon reaching confluence, the PDLCs contracted their monolayer around two pins placed 7 mm apart in the center of the well to form a cylindrical, scaffold-free construct. The constructs were histologically characterized using hematoxylin and eosin staining, alizarin red staining to detect mineralization, and immunostaining against bone sialoprotein (BSP) and scleraxis as markers of mineralized or ligament tissue formation, respectively.

Results: Scaffold-free PDLC constructs are solid and highly cellular tissues. Positive alizarin red staining and BSP expression was localized to the center of construct indicating the formation of mineralized tissue core. A ligament-like structure formed on the periphery of the engineered tissue as characterized by positive peripheral scleraxis expression.

Conclusion: Scaffold-free tissues engineered using PDLCs self-assembled to form a mineralized tissue core with a ligament-like structure on the periphery. The results of this study show that PDLCs can organize into multiple periodontal tissues. These engineered tissues could be used as a regenerative therapy to treat periodontal disease or as a model system to study periodontal tissue assembly.

A Systems Model of Human Metastatic Melanoma from Invasion to Colonization Identifies Tenascin-C as a Driver of Resistance and Emergence

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The lethal progression of metastatic melanoma is driven by refractory micrometastases. These small tumors are generally chemoresistant, and under growth arrest. Dormancy confers drug resistance and secures eventual ectopic emergence. Limited information is known in regard to drivers of metastatic emergence. Human tissue biopsies indicate that metastatic melanoma harnesses the tumor microenvironment (TME) to develop a drug resistant niche, at which point durable therapeutic responses are rare. While genetically engineered mouse models have been instrumental in identifying the impacts of melanoma driver mutations, the study of cell programs governing early ectopic seeding, survival, and emergence have been constrained by inherent complexity. To address this we have developed the first all-human microphysiological systems (MPS) model of metastatic melanoma to study key events from dissemination to colonization.

Using the MPS, an all-human organotypic skin organ has been connected via microfluidic circulation to a functional 3D human liver organoid to model critical changes in the primary and ectopic TMEs. Pathophysiologic changes in the TME occur early in primary melanoma which is marked by increased expression of the ECM protein, Tenascin-C (TNC). The presence of TNC in melanoma tumors is correlated to increased malignancy and poor patient prognosis. Invasive outgrowth of melanoma tumor spheroids is increased by melanoma derived TNC, and by stimulatory epidermal growth factor (EGF) and inflammatory ligand lipopolysaccharide. However, invasive outgrowth appears to be more readily influenced by cancer associated fibroblasts (CAFs). CAFs are the primary source of cancer ECM within primary and ectopic TMEs, and are hypothesized to create a tumor permissive ECM for outgrowth and promote tumor survival via secretion of TNC which contains intrinsic low affinity, anti-adhesive, EGF-like repeats. Preliminary studies in the MPS using high TNC expressing metastatic melanomas indicate that TNC is key to driving vertical invasion into collagen matrices. Strategies will be aimed at targeting stromal and cancer cells producing TNC to limit outgrowth and invasion within the TMEs.

Development of a Novel Topical Controlled Release System for Otic Drug Delivery

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Acute otitis media (AOM), or middle ear infection, is the main indication for pediatric antibiotic prescription in the United States, accounting for over 20 million physician visits annually. Existing treatments of systemic antibiotics and ear drops have low efficacy due to low patient compliance and the potential for harmful side effects. The overall goal of this study is to present proof-of-concept for a topical controlled release system using drug-loaded microspheres (MS) applied to the target area via a gel drop depot that may significantly improve patient care in treatment of AOM.

Fabrication of microspheres and gel was modified from previous studies. Microspheres, loaded with ciprofloxacin, an antibiotic commonly prescribed to treat AOM, were fabricated via a double emulsion procedure using poly(lactic-co-glycolic) acid. A reverse thermal, poly-N-isopropylacrylamide-based gel was synthesized via free radical polymerization. Microsphere morphology was characterized by scanning electron microscopy (SEM). Release profiles were determined via spectrophotometry and high-performance liquid chromatography (HPLC). To qualitatively analyze retention in the ear canal, fluorescently dyed gel was applied to simulated tympanic membrane (TM) within a transparent plastic ear model and evaluated by visual inspection. Ex vivo studies estimated permeation of drug across the TM. Ear canals were harvested from humanely sacrificed guinea pigs and visually inspected to confirm lack of perforation. Excised ear canals were suspended in phosphate buffered saline (PBS) and gel-MS applied to the TM. Drug concentration was determined by spectrophotometric evaluation of the PBS every 24 hrs.

SEM of ciprofloxacin-loaded MS indicates consistent morphology. Release studies show constant linear release of ciprofloxacin over 14 days. Retention studies show that the gel drop is retained on simulated TM for extended periods of inversion, suggesting that the drop will be retained during normal use. Ex vivo transtympanic testing demonstrates therapeutically relevant drug levels can be released in a controlled manner. These studies are being expanded to a chinchilla model of AOM to evaluate ability of drug to clear active infection. This drug delivery system will also be expanded to investigate topical delivery of growth factors for regeneration of perforated TM.

Bioactive Ovarian Hydrogels Provide a Novel Biomaterial for In Vitro Follicle Maturation

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Female cancer patients have a significant risk of losing reproductive function due to harsh 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 by several histological and biochemical techniques. Decellularized tissues were lyophilized, milled into a fine powder then enzymatically digested. Hydrogels were formed at 37°C after neutralizing the digested ECM. Hydrogel mechanical properties and ultrastructure were assessed using rheology and scanning electron microscopy (SEM). Secondary ovarian follicles were isolated from 16-day old female mice and encapsulated in ovarian hydrogel (OECM) droplets then cultured for six days in growth media. Bright field images were used to assess follicle diameter and morphology.

Ovarian tissues were effectively decellularized with a 91% 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 showed an increase in follicle diameter and maintained normal morphology after six days in culture. Our results indicate that OECM hydrogels can support follicle growth for at least six days in culture suggesting that this novel biomaterial could be used for IVM to obtain mature oocytes for fertilization and may be utilized as a delivery vehicle for follicle transplant.

Chondrocyte Hypertrophy and Mineralization is Controlled by Hydrogel Composition

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INTRODUCTION: Physeal cartilage exhibits a stratified cellular architecture with chondrocytes in zones of distinct differentiation states. To successfully regenerate the physis, biomaterials should support the physeal chondrocyte phenotype progression and facilitate proper interzonal signaling. Here we evaluated the effects of a hydrogel that mimics the composition of cartilage matrix (PGH) versus a popular photocrosslinkable gelatin hydrogel (GEL) on the phenotype progression of chick sternal chondrocytes.

METHODS: Polymer precursors were methacrylated in-house. The hydrogels were prepared by dissolving polymers at in HBSS, adding photo initiator, and photopolymerizing with UV-A. To evaluate effects of the hydrogels on progression of chondrocyte phenotype states, three chick chondrocyte populations were isolated from distinct regions of chick embryo sternae, encapsulated in cylindrical constructs, implanted in mice and grown for up to 8 weeks. The implants were analyzed for matrix composition and cell phenotype with histology and immunohistochemistry. To determine the mechanism driving the hydrogel effects, we investigated the hydrogel effects on gene expression of hypertrophic cells. We also evaluated whether the gelatin component of hydrogels mitigated the effects using an MMP inhibitor GM6001. Samples were collected after one week for histology, glycosaminoglycan content analysis, and PCR.

RESULTS: The GEL hydrogel showed decreased GAG for all chondrocyte populations and accelerated chondrocyte progression to hypertrophy compared to the PGH hydrogel. The PGH hydrogel augmented maintenance of prehypertrophic and hypertrophic chondrocytes in a GAG producing state while permitting development of the hypertrophic phenotype in prehypertrophic cells. Chondrocyte mineralization was completely inhibited in PGH hydrogel. Both GEL and PGH hydrogels showed downregulation of hypertrophic gene expression and up-regulation of anabolic gene expression. Comparing to its GEL counterpart, chondrocytes in PGH expressed 30 times more MMP1 and twice as much AGN. GM6001 did not down regulate hypertrophic markers. However, it increased AGN expression in GEL to the levels of untreated PGH.

Whole Eye Transplantation: Allograft Survival With Tacrolimus Immunosupression And Comparison To Syngeneic Transplantation

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INTRO: Blindness is a devastating condition affecting millions of patients. Whole eye transplantation (WET) is a potential solution. Our lab has established a viable rodent model with promising results in syngeneic transplants. To investigate allotransplantation, successful immunosuppression is necessary. Tacrolimus monotherapy is successful in rodent VCA and has possible neuroprotective effects in the central nervous system and injured optic nerve, but its efficacy in WET is unknown.

METHODS: Brown-Norway to Lewis rat transplants were performed (n=6), followed by daily intraperitoneal 1mg/kg Tacrolimus injection. Animals were examined at weeks 1, 3, 5, and 6, and compared to syngeneic transplants. Exams included Optical Coherence Tomography (OCT), clinical examination by retinal specialist, intraocular pressures, and histology interpreted by ocular pathologist.

RESULTS: Compared to syngeneic transplants, allografts demonstrated comparable corneal thickening, retinal thinning, and blood flow in the central retinal artery and vein (OCT). Intraocular pressures were normal and comparable to syngeneic transplants. On clinical examination, both groups had mild corneal anomalies, but allografts had more frequent fundus and optic nerve ischemia (moderate). Histologically, both groups had global ocular chronic inflammation, some degree of retinal degeneration, but, in contrast to allografts, syngeneic transplants actually showed consistent degeneration of the optic nerve.

CONCLUSION: This is the first study of orthotopic allograft eye transplantation and immunosuppression. Compared to syngeneic transplants, allografts had increased ischemia, but less optic nerve degeneration, without signs of rejection. Overall preservation of ocular structures is an exciting first step. With this, we can begin to explore innumerable new questions in eye transplantation.

A multi-organ microphysiological system that models dormant-emergent metastatic breast cancer progression

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Breast cancer (BrCa) mortality predominantly results from distant metastases that are not visible at diagnosis and escape initial therapies to lie as dormant micrometastases for years. To study the behavior of micrometastases – how they escape initial treatments and then awaken from a dormant state – we utilize an all-human ex vivo hepatic microphysiological system (MPS). The functional liver unit, comprising hepatocytes and non-parenchymal cells in a 3D microperfused culture format, is stable over a month and mimics the dormant-emergent metastatic progression observed in human patients: (i) a subpopulation of BrCa cells spontaneously enter dormancy, (ii) cycling cells are eliminated by standard chemotherapies, while quiescent dormant cells remain, and (iii) chemoresistant dormant cells can be stimulated to emerge. Multiplexed analysis of the circulating medium (system blood mimic) revealed molecular signatures which discriminated between livers containing dormant or growing (either primary or secondary emergence) BrCa cells. Interestingly, tumor dormancy correlated with a signaling fingerprint reflective of non-inflammatory quiescence, whereas outgrowing nodules moved in lockstep with inflammatory signatures. A second premise explored involved the potential role of “leaky gut syndrome” in driving emergence. For this, we created a multi-organ MPS supporting the liver module in communication with an immune-active gut module. Upon stimulation of this platform with LPS, synergistic production of several cytokines was observed; including CXCR3 ligands that we show can drive tumor cells to emerge from quiescent dormancy. Ultimately, this MPS provides unprecedented insights into the biology of quiescent tumor dormancy and inflammation-mediated tumor outgrowth.

Regenerative Potential of Various Soft Polymeric Scaffolds in the TMJ Condyle

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Purpose: Biodegradable polymeric scaffolds have been used for tissue engineering approaches and can also be employed to regenerate the TMJ joint tissues. Synthetic acellular polymeric Poly (glycerol sebacate) (PGS) scaffolds and natural scaffold made from gelatin are polymeric scaffold sponges that may provide a substrate for cell infiltration and remodeling. Here, we studied the regenerative potential of both scaffolds along with a bioactive signal, Magnesium in a novel fibrocartilage defect model in the goat MCC. Furthermore, in a departure from the pig model, we are developing the goat as a repeatable surgical model with easy access into the joint space on skeletally mature animals.

Methods: By using this model, bilateral osteochondral defects were created in the mandibular condyle of mature female Spanish Boer goats. A 1mm diameter drill was used to create a trough defect on the articular surface. We evaluated 4 different groups: 1) an empty control without an implant, 2) PGS with Mg ions, 3) gelatin with Mg ions, and 4) gelatin with both Mg ions and trimagnesium phosphate (TMP) powder. Goats were allowed to heal for 3 months, and then the tissues were harvested.

Results: The empty control showed a thin fibrous layer growing within the defect. Both the PGS and gelatin sponge groups yielded a cartilage layer with glycosaminoglycan and Collagen Type II, along with robust regeneration of fibrous layer as seen by cellular infiltration and collagen in the defect. The TMP in the gelatin did not degrade, and seemed to hamper healing.

Conclusion: These results suggest that both synthetic and natural sponges are capable to providing a template for new tissue growth in the MCC of the TMJ. Furthermore, this study is the first to use the goat as an in-vivo TMJ model.

Role of ECM-associated IL-33 in Functional Cardiac Repair

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Fibrosis occurs when cardiac myocytes damaged by ischemia are replaced by fibroblasts that produce excessive extracellular matrix (ECM). Increased stiffness of fibrotic myocardial tissue leads to heart failure. Decellularized ECM has been used as both a solid sheet and an injectable hydrogel to promote constructive remodeling of cardiac tissue. The precise mechanisms by which ECM directs cardiac tissue repair are only partially understood, but the ability of ECM to activate macrophages toward a pro-remodeling phenotype is thought to play a role. The recent identification of matrix bound nanovesicles (MBV) embedded within the ECM provides potential insight into the mechanisms behind the inductive properties of ECM materials. We have discovered that MBV derived from ECM are a rich source of extra-nuclear interleukin-33 (IL-33). IL-33 is a member of the IL-1 family of cytokines and has been classified as an "alarmin", though there are conflicting reports of pro- versus anti-inflammatory effects. IL-33 is critical to control of early inflammation and tissue repair after acute chemical injury in the lungs and prevents chronic rejection-associated transplant fibrosis.

MBV can directly activate macrophages towards a pro-remodeling phenotype; thus, it is plausible that the immunomodulatory effects of MBV could facilitate the resolution of the inflammatory response associated with cardiac ischemia and hasten constructive cardiac repair. MBV were isolated from wildtype (il33 +/+) and IL-33 deficient (il33 -/-) mice to identify the potential role of IL-33 in immunomodulation and remodeling of cardiac tissue. MBV from wildtype (il33 +/+) mice activated macrophages toward a pro-remodeling M2-like phenotype through a non-canonical ST2 receptor-independent pathway, while il33-/- MBV promoted a pro-inflammatory M1-like phenotype in st2-/- macrophages. In addition, the secretome of st2-/-macrophages treated with wildtype MBV promoted myogenesis in vitro. These data suggest that IL-33 encapsulated within MBV is critical to promote M2-like activation of infiltrating immune cells and supports downstream tissue repair.

Biodegradable Conduits for Long Gap Peripheral Nerve Repair in a Non-Human Primate Model

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The gold standard of care for peripheral nerve injuries is autografting, which may result in donor site morbidity, including loss of sensation and function, and multiple incisions leading to longer surgeries and increased risk for infection. In patients with multiple nerve injuries, obtaining adequate donor nerve tissue can be challenging. We are investigating an “off-the-self” biodegradable poly(caprolactone) nerve conduit embedded with glial cell line-derived neurotrophic factor (GDNF) as a promising alternative to autografting.

A 5-cm defect was created on the distal median nerve of non-human primates (NHPs). The gap was repaired with either an autograft, decellularized human allograft, PCL conduit with GDNF, or an untreated empty PCL conduit. The gradual return of hand function was assessed using a modified Klüver board, tested at a pre-operative baseline and regularly assessed through a 1-year period. The percentage of correct pinch retrievals was recorded. Compound nerve action potentials (CNAP), nerve conduction velocity (NCV), and compound motor evoked potentials (MEPs) were recorded using intraoperative electrophysiology. Histological analysis determined neurofilament and Schwann cell density. A biopsy of the abductor pollicis brevis (APB) muscle, which is innervated exclusively by the median nerve was taken at 1-year post-procedure and stained to assess muscle atrophy.

The PCL/GDNF and autograft treatment groups trended towards improved functional return compared to the untreated PCL group at 1 year versus baseline. The decellularized group had higher neurofilament density than the PCL/GDNF group. Nerve conduction was present in the APB of the PCL/GDNF and decellularized nerve groups suggesting the nerve was able to regenerate across the 5-cm gap and reinnervate the APB. Future work will focus on concluding NHP trials clinical translation of this research.

Porcine Infected Partial-Thickness Burn Wound Model

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BACKGROUND: Burn injuries a common source of trauma worldwide and partial-thickness burns often become infected as they are typically left to heal secondarily. This is especially prevalent in the military, where soldiers serving in overseas have a high prevalence burn wound infections secondary to MRSA and other pathogens. We have developed a porcine infected partial-thickness burn wound model to eventually test a novel polysaccharide compound for its ability to sterilize infected burn wounds.

METHODS: 16 burn wounds were created on the back of a female Yorkshire pig using a brand heated to 200oC and applied with 1kg of force for either 40s, 10s, 5s, or 2s (n=4 per group). Biopsies were taken 30 minutes after burning for histology and wounds were dressed with bacitracin, Adaptec, Opsite, cotton pad, and spandex jacket. This pig was taken back to the OR on post-operative day two for debridement of necrotic skin. Dressing changes were performed three times a week for 14 days and wound healing was observed using photographs and tracings. 24 burn wounds at 10s were created on the back of a second female Yorkshire pig and received either no treatment, scraping with MRSA, or injection of MRSA (n=8 per group). Wounds were dressed the same as the first pig and biopsies were taken at each dressing change for culture. Both pigs were sacrificed 15 days after surgery.

RESULTS: 40s burns were full-thickness while burns at all other times were partial-thickness at varying depths. Burns for 5s and 2s were superficial partial-thickness and healed by day 14, while 10s were moderate-to-deep partial-thickness and still healing at day 14. Thus, is was determined that 10s was the appropriate burn length. In the second pig, burning for 10s again gave moderate partial-thickness burns and all wounds were infected by post-operative day four. Cultures revealed a poly-microbial infection with likely MRSA and Pseudomonas and wounds were still healing at day 14.

CONCLUSION: Applying our brand 10s provides a reliable partial-thickness burn. Scraping MRSA can cause an infection of this burn but is difficult to control when applying this in solution over the convex back of the pig. Therefore, future experiments done using this model will require polyurethane wound chambers to adequately isolate each wound.

A Novel Bioreactor Model for the Study of Human Aortic Disease

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Objective: To develop a model to investigate aortic smooth muscle cell (SMC) biology and their link to extracellular matrix (ECM) remodeling in the context of ascending aortic disease utilizing a novel, patient-specific, bioreactor-based 3-dimensional dynamic culture.

Methods: SMCs from ascending aortas were isolated, expanded, seeded onto tubular poly (ester carbonate urethane) urea (PECUU) scaffolds, and placed into bioreactor culture for 4 weeks. Control scaffolds were seeded and maintained in non-dynamic culture conditions for 24 hours (T0). Scaffolds were analyzed for SMC phenotype retention via RT-qPCR for Acta2 and Calponin and immunostaining for alpha-smooth muscle actin (SMA) and calponin. ECM production was assessed using RT-qPCR for Elastin, Collagen 1, and Collagen 3 along with Masson's trichrome, Picrosirius Red, multiphoton microscopy, and Sircol and Fastin assays. Gene expression for ECM modulating genes (Fibrillin 1, Fibulin 5, MagP1, and Vcan) was also investigated.

Results: Bioreactor cultures showed retention of SMC phenotype as indicated by similar levels of SMA and calponin relative expression when compared to T0. Scaffolds also demonstrated increased collagen and elastin production after 4 weeks in dynamic culture. In concert multiphoton microscopy detection of collagen's second harmonic generation (SHG) also revealed increased signal in bioreactor-cultured scaffolds. RT-qPCR data showed up-regulation of Elastin, Collagen 1, Collagen 3, Fibrillin 1, Fibulin 5, MagP1, and Vcan (all $p < 0.017$). Levels of soluble collagen and elastin content were increased from $0.44 \pm 0.07\mu g$ to $1.49 \pm 0.15\mu g$ ($p < 0.016$) and $11.63 \pm 1.26\mu g$ to $25.65 \pm 2.03\mu g$ ($p<0.031$), respectively.

Conclusion: We present a novel bioreactor-based 3-D culture model that is capable of stimulating collagen and elastin synthesis from ascending aortic SMCs derived from patients with varying degrees of aortic disease. Future investigations will focus on studying patient-specific differences in ECM production and remodeling to shed light onto the underlying mechanisms leading to aneurysm formation.

ECM hydrogel injection for the treatment of stroke: Time course comparison of hydrogel retention and phenotypic characterization of invading cells

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Stroke is the leading cause of adult disability and a significant effort is under way to develop therapies to repair the damaged tissue. The loss of function after stroke is caused by the death of neurons, leaving behind a tissue cavity filled with extracellular fluid (ECF) and cell debris. Biomaterials composed of mammalian extracellular matrix (ECM) promote constructive tissue remodeling with minimal scar formation. At ECM concentrations that have similar rheological properties as brain tissue, the biomaterial exists in fluid phase at room temperature, while forming hydrogels at body temperature. ECM with different concentrations (0, 3, 4, 8 mg/mL) was injected into the lesion cavity after stroke to support endogenous repair mechanisms. Retention and gelation of the ECM, as well as host cell invasion and phenotype was analyzed at 1, 14 and 90 days post-injection using immunohistochemistry. Complete retention of ECM hydrogel within the cavity occurred at concentrations >3 mg/mL, with extensive diffusion into the host tissue at lower concentrations. A significant host cell invasion into the ECM hydrogel was seen at 1 day post-injection, with an average of over 350,000 cells invading in the 8 mg/mL concentration. As the acute inflammatory response was replaced with an ECM remodeling phase at later time points, there was a significant decrease in the total number of cells invading the biomaterial. Initial invading cells were of a microglia and macrophage phenotype and followed specific trails into the ECM biomaterial along topological features conducive to cell migration. The follow-on cells were neural and oligodendrocyte progenitor cells, which are essential for repopulation of the neural tissue. This characterization demonstrates that an ECM hydrogel can be readily injected and retained within the lesion cavity, while promoting a continued endogenous repair response. A behavioral study is necessary to evaluate the therapeutic efficacy of this approach.

Cardiac Extracellular Matrix Aging Impacts Macrophage Phenotype and Function

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Clinical interventions following myocardial infarction (MI) are limited in efficacy due to the limited proliferative capacity of post-mitotic, adult mammalian cardiomyocytes, which are depleted in large quantities during MI events. However, potent cardiac regeneration in neonates and limited cardiomyocyte proliferation in aged individuals following MI has been observed. This neonatal cardiac regeneration is not holistically dependent on non-terminally differentiated cardiomyocytes but is also dependent on the macrophage populations present in the tissue. Cardiac macrophages are derived from three unique sources and colonize cardiac tissue during distinct periods of development. However, the factors governing the maintenance and recruitment of these macrophages in aged individuals remains unclear. The local microenvironment composed of extracellular matrix (ECM), growth factors, chemokines, and numerous additional soluble factors plays a substantial role in determining macrophage development and phenotype. Thus, this study sought to examine the potential role cardiac ECM aging plays in altering macrophage phenotype and functionality. A decellularization protocol was optimized to isolate murine cardiac ECM. Biochemical and DNA quantification assays were performed to confirm decellularization. Bone marrow derived macrophages isolated in culture were exposed to either young or aged cardiac ECM degradation products. Following 24 hour ECM exposure, macrophage nitric oxide production and gene expression levels were assessed at baseline and following exposure to canonical M1 or M2 polarizing cytokines. Macrophages exposed to aged cardiac ECM degradation products exhibited increased nitric oxide production compared to young ECM-exposed groups, a functional response exhibited in pro-inflammatory macrophages. Additionally, qRT-PCR analysis revealed several alterations in gene expression following ECM exposure with subsequent cytokine polarization. These results indicate that macrophages exhibit differential responses to young and aged cardiac ECM degradation products in vitro.

Development of a Seeding Device for Bulk-Seeding of Cells into a Long "Human-Sized" Scaffold for Tissue Engineered Vascular Grafting

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Introduction

The fabrication of tissue engineered vascular grafts (TEVGs) for the treatment of vascular disease often incorporates cells into scaffolds through seeding. Most work to date has focused on TEVG implants into small animal models. The generation of cell-based, "human sized" (~4 mm inner diameter, ~150 mm length) TEVGs for testing in large animal models or eventual clinical translation requires scaling-up of both the scaffold and the seeding system. We developed and performed a validation of a next-generation seeding device that allows even, longitudinal seeding of large scaffolds for the fabrication of "human-sized" TEVGs.

Methods

A novel seeding device was developed that deposits a cell suspension injected through a computer-controlled translating lumenal diffuser with axial rotation and steady trans-scaffold vacuum pressure. Cells were seeded into a large (~4 mm inner diameter, ~150 mm length) scaffold fabricated from poly(ester-urethane) urea (PEUU) with an inner porous layer for cell retention and an outer electrospun layer for mechanical resilience. The seeded "construct" was kept in static incubation for a brief period (6 hours) to allow for cell adherence. The construct was then segmented and neighboring portions were either analyzed immediately or after 48 hours of dynamic culture. Validation of proper seeding was determined using MTT assays and histological nuclear staining.

Results

Seeding with approximately 100M mesenchymal stem cells was performed on a ~150 mm PEUU scaffold. Cellular activity was detectable within the construct directly after seeding and increased ~3-fold following 48 hours of dynamic culture. Histological sections from the construct revealed disperse cell seeding around the circumference with uniform seeding through the thickness in areas of the porous scaffold layer.

Discussion

The linear translating diffuser based, rotating vacuum seeding device is capable of distributing cells around the circumference and through the thickness of a "human-sized" TEVG construct based on both histology and MTT assays. Additionally, the increase in cellular activity demonstrated by the MTT assay suggests a marked increase in cell viability as a result of the 48-hour dynamic culture period. Future work will optimize the density of seeded cells for implantation into a large animal model and eventual translation for human use.

In Vitro Optimization of a Controlled Release Cysteamine Eye Drop

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Nephropathic cystinosis is an autosomal recessive disease characterized by the mutation of the CTNS gene and its protein product cystinosin. Cystinosin functions to transport cystine out of cell lysosomes. Lack of cystinosin function causes intracellular accumulation of cystine which crystallizes and damages organ tissues including the kidney, pancreas, thyroid and eyes. Cystine accumulates in all the ocular tissues but is most easily evident as crystal deposits in the cornea that lead to debilitating photophobia, corneal erosion, and can lead to blindness. Corneal cystine crystals are treated by hourly administration of topical cysteamine eye drops. The eye drop formulation requires a high concentration of cysteamine per drop to account for its instability as it is easily oxidized to inactive cystamine. The strict dosing regimen and high concentration of drug per drop make this treatment inconvenient and painful for patients leading to almost universal non-compliance. A suitable controlled release formulation may help decrease the number of daily eye drops and prolong the effect of treatment. Our group has developed a thermoresponsive, gel-based eye drop that contains cysteamine-loaded. The thermoresponsive hydrogel matrix is administered as a liquid drop and is retained within the conjunctival cul de sac. This study describes the development of a cysteamine microsphere formulation optimized using several emulsion-based techniques. To achieve clinically relevant drug levels, microsphere formulations were fully characterized in vitro for relevant physical and chemical properties. The candidate formulation produced release kinetics for up to seven days with a burst release on the first day. The release profile within the first day was also determined. To determine the achievement of clinically relevant drug levels, future studies involve the stability of drug formulation and in-vitro efficacy using cystinosin knockout fibroblasts.

Three-Dimensional Nanodevice Arrays for Exploring Electrophysiology in Engineered Excitable Microscale Tissues

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Studies of electroactive tissue have been carried out using a variety of recording techniques, including glass micropipette patch-clamp electrodes, voltage-sensitive dyes, multielectrode arrays (MEAs), and planar field-effect transistors (FETs). However, multisite, simultaneous, three-dimensional (3D), and long term electrophysiological investigation of an engineered tissue has not been possible using these technologies. Patch clamp is limited by recording sites, while voltage or Ca2+ sensitive dyes are potentially limited by toxicity to the cells. MEA and planar FET are confined to 2D, rendering 3D electrical recording challenging. We report an approach to record electrical signals from excitable tissues in 3D with high spatiotemporal resolution. A self-rolling 3D nanodevice array (SR-3DNA) composed of graphene-based FETs was designed to encapsulate microscale tissue and enable continuous recording of electrical signals. The SR-3DNAs were fabricated on a sacrificial layer and SU8 polymeric support. Graphene was synthesized by Cu foil catalyzed low-pressure chemical vapor deposition, validated by optical microscopy and Raman spectroscopy, transferred to the surface of the SR -3DNA, and patterned by photolithography followed by reactive ion etching. Source and drain contact electrodes for the nanosensors were deposited by electron beam evaporation and attained intrinsic residual stress during the process. Upon sacrificial layer etch the residual stress was released, and SR-3DNAs spontaneously assembled in 3D. By varying the thickness of SU8 support, the diameter of the SR-3DNAs was tailored to fit each engineered microscale tissue. The FEA simulation confirmed the controlled residual stress driven self-rolling behavior of the SR-3DNAs. The simulated radius of curvature and the experimental results were highly correlated. Graphene-based FETs demonstrated characteristic ambipolar water-gate response. Furthermore, the SR-3DNAs were unrolled to encapsulate electroactive microscale tissues. Using this platform, electrophysiological studies can be conducted to directly monitor the development of electrical activities in engineered microscale tissues over time.

Matrix-bound Nanovesicles as Regulators of Achilles Tendinopathy during Healing

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Tendinopathy is an overuse injury that has both an inflammatory and degenerative component and is not well understood. Tendinopathy accounts for 65% of Achilles tendon disorders. The natural wound healing process in the Achilles tendon promotes the formation of scar tissue that is associated with impaired mechanical strength, matrix content, and matrix organization. There is no consensus regarding the best method of treatment for tendinopathy. Bioscaffolds composed of mammalian extracellular matrix (ECM) have been widely used clinically to promote constructive and functional tissue remodeling in a variety of tissues, including the musculoskeletal tissues. ECM-mediated constructive tissue remodeling has been attributed to bioscaffold degradation and the associated release of bioactive components including matrix bound nanovesicles (MBV). MBV have been shown to down-regulate an inflammatory phenotype in macrophages, promote neuronal stem cell differentiation, and promote collagen crosslinking.

In the present study, tendinopathy was induced by collagenase injection in a rat model and treated with MBV isolated from different ECM. MBV derived from dermis ECM with and without a proteinase K step, and MBV from UBM with and without proteinase K were used as treatment groups along with sterile saline and PBS control groups. MBV-bound lysyl oxidase (LOX) exists in multiple isoforms and has robust functional activity responsible for collagen cross-link formation and stabilization of collagen fibrils. Additionally, proteinase K strips the surface receptors of MBV for LOX and serves as a control for the presence of LOX on MBV. Understanding the associated release of MBV through the homeostatic process of LOX becoming incorporated into the ECM of native tissues would allow a regenerative medicine approach to enhance the biomechanical properties of the injured tissue. The use of MBV alone as a treatment for Achilles tendinopathy is an attractive potential strategy that capitalizes upon naturally occurring processes.

Spark Plasma Sintered Titania for Potential Application as a Keratoprosthesis Skirt

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The potential application of titania materials as artificial corneal implant skirts relies heavily on their mechanical properties and and biocompatibility. This study was aimed at optimizing the selection of raw powder and sintering conditions for titania ceramics. Titania compacts were synthesized from five raw powders, denoted as Altair, Inframat, Alfa, Materion and Amperit, respectively, by spark plasma sintering using different sintering parameters. The nanoindentation results indicated that among the five types of titania samples sintered at 1100 deg. celcius, the Inframat pellets possessed the highest Young's modulus and hardness. Culture of human corneal stromal fibroblasts on the sintered sample surfaces showed that comparably high cell viability and proliferation were observed on all titania samples except Amperit compared to positive control. Furthermore, cells cultured on Inframat titania sintered in the temperature range of 900–1300 deg. exhibited viability and formation of focal adhesion complex similar to those on control, and those prepared at 1100 deg. had significantly higher cell proliferation indices than control. In conclusion, Inframat titania consolidated at 1100 deg. by SPS was the best formulation for the preparation of mechanically strong and biocompatible Keratoprosthesis skirt.

Controlling Release of Antibodies from Skin Transplant using Bioaffinity Coassemblies

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The purpose of this work was to characterize a coassembly platform based on self-assembling peptide (SAP) for controlled release of antibodies. The design centers on the novel construct pG_EAK, in which a truncated protein G, consisting of only the Fc-binding C domains is fused with repeating units of the amphiphilic sequence AEAEAKAK, or 'EAK'. Intermixing pG_EAK with molar excess of EAK peptides generates coassemblies with multivalent IgG-binding sites. We sought to determine the extent to which IgG can be concentrated in skin transplants with limited systemic exposure. pG_EAK was expressed and purified from E. Coli transformed with a plasmid with the nucleotide sequences encoding both pG and EAK. Efficiency of pG_EAK and EAK coassembly was analyzed using SDS-PAGE. It was determined that the coassembly was most efficient at 1:400 molar ratio, in which >90% of pG_EAK was incorporated. Loading of antibodies into the coassembly was examined using a fluorescein-labeled IgG as model biotherapeutic. Three times more fluorescence was observed from IgG retained in the coassembly compared to IgG loaded in EAK assembly alone. The local retention of pG coassembly-immobilized IgG was tracked in vivo using a near infrared dye-labeled antibody (IgG800). SC injection of IgG800 co-administered with pG_EAK and EAK on the back of mice resulted in detectable fluorescence at the site for at least two days; while injection of the antibody alone resulted in complete loss of fluorescence after one day. Localization of IgG800 at the injection site corresponded to fluorescence in the liver, spleen and lymph nodes, in which lower signals were detected in these organs when the antibody was injected with pG_EAK and EAK. A murine skin allo-transplant model was used to test the stability of the IgG in skin transplantation. The same pattern was seen at skin transplant sites: IgG800 remained underneath the allografts longer (for 6 days) and lower signals were detected in the liver, spleen and lymph nodes relative to the antibody formulated in saline. The data indicate that coassemblies of pG_EAK and EAK retained IgG at SC tissues for extended duration.

Radical Inflammatory Species-Decellularized ECM Promotes Antioxidant, Pro-Healing Host Response in Muscle Injury

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Extracellular matrix biomaterials have been shown to elicit a timely shift from pro- to anti-inflammatory immune phenotypes. Natural pro-inflammatory immune responses involve the production of radical nitrogen and oxygen species, which cause vasodilation, immune-protection and angiogenesis. Radical modification of biomolecules, including nitrosylation and oxidation of proteins and lipids, within wound environments trigger antioxidant and anti-inflammatory responses from immune cells. Decellularization or modification of extracellular matrix biomaterials, which include structural proteins as well as remnant cellular lipids and proteins, with radical inflammatory species (RIS) could trigger an enhanced anti-oxidant, anti-inflammatory response. Small intestine submucosa extracellular matrix scaffolds were decellularized using reactive nitrogen species (RNS), reactive oxygen species (ROS), RNS+ROS or peracetic acid (PAA) as a control. All ECM were sufficiently decellularized with no difference in DNA content. RIS-ECM exhibited reductions in detected hydroxyproline and glycosaminoglycan content compared to PAA controls. They also showed increases in immune-histochemical staining for protein oxidation and nitrosylation markers. In vitro assessment of the immune response to ECM degradation products was conducted using a murine bone marrow-derived macrophage population. Macrophages exposed to RIS-ECM showed enhanced anti-inflammatory (arginase-1) and anti-oxidant (HO-1) immunolabeling, as well as reduced phagocytosis and nitric oxide production compared to controls. These ECM scaffolds were implanted into a mouse abdominal muscle defect model for 7 days, proven to be a critical time-point in responses to biomaterials. Mice implanted with RIS-ECM showed increased immunofluorescence labeling for arginase-1 and HO-1 compared to controls. These results suggest that modification of ECM bioscaffolds with radical inflammatory species enhances the anti-inflammatory and anti-oxidant immune response. These responses could be beneficial for a variety of wound healing applications, especially in oxidative diseases such as chronic ulcers and ischemic injury.

Determining and mimicking the role of mesenchymal stem cells within an implanted elastomeric vascular scaffold

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Significance
Cardiovascular disease is the number one cause of death in the US and treatment of this disease often requires revascularization with a graft. Stem cell-based tissue engineered vascular grafts (TEVGs) show promise, but translatability of these grafts is limited due to regulatory restrictions resulting from in vitro cell expansion.

Objective
The purpose of this study was to observe the time-course of stem cell retention in order that artificial mesenchymal stem cells (artMSCs) could be designed to replicate the duration and paracrine activity of stem cells but without the same regulatory limitations.

Methods
To determine the duration of signaling activity of the cells within our scaffolds in vivo, bilayered poly(ester urethane)urea (PEUU) scaffolds seeded with mesenchymal stem cells were implanted as infrarenal aortic grafts in Lewis rats. After 1 or 4 weeks in vivo, the grafts were explanted and analyzed using immunofluorescent chemistry for human nuclear antigen to detect any remaining seeded cells. To determine the time-course of artMSC release, for comparison with the duration of MSC retention, bioactive factor-loaded artMSC were seeded into PEUU scaffolds, incubated in a saline solution for 21 days, and sampled for releasate daily. Total protein in each sample was then measured using a bicinchoninic acid assay. Potential toxic byproducts were monitored by incubating releasates with cells, and performing a LIVE/DEAD assay. To test for expected retention of artMSCs in vivo, fluorescein isothiocyanate (FITC)-loaded microspheres were seeded into scaffolds and implanted into our rat model. The scaffolds were then explanted after 3 days, sectioned, and imaged for FITC fluorescence.

Results
All TEVGs were observed to be patent at explant. Immunostaining for HNA was positive at both 1 and 4 weeks post-implant, with no significant decrease in the percentage of seeded cells that were HNA positive from 1 to 4 weeks. A linear release of total protein from the scaffolds over a 10 day period was observed. 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 for 3 days.

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

Degradation of engineered polyurethane heart valves in a mechanically demanding environment with variable polyester or polycarbonate soft segments

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Valvular heart disease (VHD) is a major source of morbidity with approximately 290,000 valve replacement surgeries worldwide each year [1]. Current technologies include mechanical or bioprosthetic heart valves, but these devices are limited due chronic anticoagulation therapy and limited durability, respectively. Tissue engineered heart valves (TEHVs) [2] attempt to overcome these limitations by employing biodegradable scaffolds that will be augmented by the endogenous tissue growth paradigm to form autologous leaflets. Understanding the time for transition from polymer leaflets to autologous tissue leaflets is required for proper stress transfer in order to avoid mechanical failure or fibrosis. Biodegradable polyurethanes, such as poly(ester urethane)urea (PEUU) and poly(carbonate urethane)urea (PCUU), provide the ability to tune the degradation profile of a tissue engineered scaffold based on the concentration of polyester and polycarbonate soft segments [3]. These soft segments can be combined using three mixing techniques: chemically bonding the polyester and polycarbonate segments into the polymer backbone (during synthesis), physically bending the PEUU and PCUU polymers in a solvent (during solvation), or electrically co-spinning the PEUU and PCUU solutions using two streams (during processing). The objective of this study was to evaluate the in vitro degradation profile of these three mixing techniques in a dynamic biomechanical environment in order to de-risk preclinical, chronic valve replacement studies. Electrospun heart valve scaffolds were fabricated from each polymer mixing technique and were subjected to accelerated degradation in a pulsatile flow loop for up to two weeks. Degradation resulted in differences in scaffold mass and thickness loss, qualitative macro- and micro-structural changes, and decreased viscosity with the chemically bonded polymer mixture degrading fastest.

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Dual Method Verification of Adipogenesis in Cultures Containing an Adipose Derived Delivery System

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Musculoskeletal injuries inflicted by wars, congenital deformities, tumor resection, and general traumatic injuries often require soft tissue reconstruction. Damages to the soft tissue can affect cosmetic appearance, hinder function, and alter emotional well-being. Autologous adipose grafting is a safe, resourceful, and minimally invasive option that has gained tremendous momentum in clinical practice. However, results can be unpredictable due to graft resorption rates reaching as high as 90%. These limitations serve as motivation for the development of new therapies to regenerate adipose tissue. Scaffolds from adipose-derived extracellular matrix (AdECM) have shown potential in its ability to generate new adipose tissue. The goal of this study was to evaluate bioactivity of a composite adipose-derived delivery system (CADDS) containing dexamethasone encapsulated in polymeric microspheres to stimulate adipose growth in vivo. Poly(lactic-co-glycolic) Acid (PLGA) (50:50) was used to encapsulate dexamethasone into microspheres (Dex MS) as a delivery system. Dex MS bioactivity was evaluated using an in vitro adipose-derived stem cell (ASC) culture model. Varied concentrations of Dex in adipogenic media, from 0 to 800 nM, were prepared using Dex MS, according to the loading capacity. Cultures were maintained for 2 weeks. Alkaline phosphatase activity (ALP) was assessed using assay kit. Six-week old Fisher 344 immunocompetent rats were chosen to appropriately assess adipogenic potential of CADDS biomaterial in vivo. Rats were divided into 4 groups receiving subcutaneous bilateral injections of hydrated AdECM containing either (1) 300 μg dose of SW Dex MS, (2) 300 μg dose of DW Dex MS, (3) Empty MS, or (4) saline only. A 6-week time point was chosen for evaluation of biomaterials in regard to adipose tissue formation, volume retention, and immune response. In vitro results showed increased amounts of ALP intracellular activity in groups with higher Dex MS concentration. The trend of ALP intracellular activity in ASCs decreased as Dex MS media concentration decreased across the six groups of 800, 400, 200, 100, 50, and 0 nM. Based on these results, CADDS bioactivity is confirmed. In vivo results indicate enhanced volume retention. Weekly photo imaging shows raised skin at the injection site indicating implant survival with minimal migration in groups containing dexamethasone. Conversely, evidence of implant survival from groups without Dex MS required manual touch to confirm suggesting low retention rates. Histological analysis is underway. As a pilot study, the current outcomes show CADDS as a potential biomaterial to address the challenges in adipose tissue reconstruction.

Combining a Nerve Specific 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 (PNSECM) hydrogel has been shown to increase constructive remodeling of injured peripheral nerves, and this study aims to show that this beneficial effect can be further increased over current standards by incorporating post-surgical therapies in a rat sciatic transection model. This rationale is based on exercise therapies having shown to increase nerve recovery independent of other therapies. This study also aims to show the efficacy of the PNSECM nerve gel in aged animals and observe potential changes in the mechanism as a result of the aging immune being known to function differently. Data will be collected up to 12 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 back to full using kinematic tracking. Surgeries for the rat sciatic transection model have begun and preliminary data will be included when available. We are conducting this study to further the creation of a multi-faceted therapy to regenerate peripheral nerves more effectively and also demonstrate applicability of this product in an aged population.

Fluorescein Angiography Used to Study Retinochoroidal Blood Flow in Whole Eye Transplantation

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Purpose
Approximately 39 million globally suffer from blindness. Whole eye transplantation (WET) has the potential to provide a viable optical system to patients with irreversible vision loss. Previous imaging studies have suggested that retinal blood flow can be established in transplanted eyes in our orthotopic vascularized whole eye transplant model in the rat. Given that retinal viability is crucial to vision, we sought to investigate the structural integrity of retinochoroidal vasculature after WET with fluorescein angiography (FA).

Methods
Exams under anesthesia (EUAs) and color fundus and FA imaging were performed in right eyes of 7 Brown Norway rats prior to transplantation. The eyes were transplanted to Brown Norway recipients (n=7) and transplanted and contralateral eyes underwent the same testing at 1 and 3 weeks after WET. Ophthalmologists with retina specialization performed EUAs, evaluated images and documented retinochoroidal findings.

Results
FA imaging revealed that all transplanted eyes exhibited complete arterial, venous and choroidal filling at 1 week after WET. Imaging of 4 of 7 transplanted eyes at 3 weeks postoperatively confirmed complete filling of retinochoroidal vasculature; the remaining 3 transplanted eyes had corneal opacities that prohibited imaging. There was no leakage in the retinal vasculature in transplanted eyes at 1 and 3 weeks after WET. Mild focal choroidal hyperfluorescence was noted in of 3 of 7 animals. 5 of 7 transplanted eyes exhibited mild to moderate retinal vessel narrowing. No abnormal optic disc hyperfluorescence was seen in the transplanted eyes at either time point.

Conclusions
FA results have confirmed retinochoroidal blood flow in a rat model of WET. In all transplanted eyes, complete vascular filling and an absence of retinal vessel leakage were noted, the latter indicating that the structural integrity of inner blood retinal barrier can be maintained after WET. The mild choroidal hyperfluorescence and vascular attenuation observed in this study will be investigated in future studies.

Fluorescent Tagging of Interleukin-4 for Visualizing In-Vivo Release from Coated Polypropylene Mesh

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Polypropylene mesh is commonly used for the repair of tissue but is associated with complications. Excised mesh-tissue complexes from patients experiencing a complication are characterized by abundant pro-inflammatory macrophages. Macrophages, however, are plastic cell types with phenotypes along a spectrum of pro-inflammatory and pro-remodeling/anti-inflammatory extremes. It has been demonstrated that modulation of macrophage phenotype during initial stages of healing could prevent chronic inflammation, improving downstream outcomes. Previous work has shown that an IL-4 eluting coating for polypropylene mesh initially polarizes macrophages to the pro-remodeling/anti-inflammatory phenotype, resulting in mitigation of the foreign body reaction downstream. However, timing and duration of the in-vivo immunomodulatory release of IL-4 (and its effect on macrophage phenotype transition) is important for timely shift to anti-inflammatory phenotypes and eventual resolution of inflammation. To customize the release of IL-4 in a spatial and temporal way, we aim to use fluorescently tagged IL-4 with live-animal in-vivo imaging in animals implanted with coated mesh. Successful visualization of IL-4 release will be important for eventual varying of release profiles in order to correlate coating patterns to downstream integration outcomes.

IL-4 was subjected to fluorescent labeling with Alexa Fluor 594. To provide the most relevant in-vivo release profile, it is important that the fluorescently tagged protein maintain bioactivity. IL-4 polarizes macrophages to a pro-remodeling phenotype with increased arginase-1 production; therefore, tagged IL-4 vs untagged IL-4 was supplemented into the media of naïve macrophages. The in-vitro culture assay showed that tagged and untagged IL-4 produced equivalent levels of increased arginase-1 when compared to macrophages that were cultured in media without supplementation, (i. e., the fluorescent tag doesn't affect bioactivity). Finally, tagged IL-4 was loaded into a dermatan sulfate-chitosan layer-by-layer coating of polypropylene mesh using previously established protocols and imaged. Confocal imaging showed a uniform signal in the red channel of mesh coated with fluorescently tagged IL-4, indicating incorporation of the tagged protein into the coating. In-vivo implantation of this tagged mesh will allow daily live-animal imaging of the same animal until loss of signal so that release profiles can be manipulated and then correlated to downstream outcomes.

Multiplexing antibodies in vivo using Z15_EAK, an Fc-binding gelation module

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“Z15_EAK” was characterized to be a novel antibody delivery hydrogel for extending local drug concentrations. When injected into subcutaneous tissues, the gel forms a scaffold in which IgG molecules are retained via the bioaffinity domain Z15. The domain is a miniaturized version of the Fc-binding Z domain derived from protein A. The truncated Z15, with molecular mass at 3.8 kDa, is expected to have low immunogenicity, thereby raising the prospect of translating the technology into clinical applications. An amphiphilic sequence (AEAEAKAK) sequence is fused to the Z15 domain to generate the peptide Z15_EAK, which can be incorporated into the self-assembling peptide EAK16-II [AEAEAKAK]2.

Spectroscopic and microscopic data suggested that Z15_EAK can be incorporated into EAK16-II gel while resulting in a mixed α-helix and β-sheet composite as expected. When probed with fluorophores Congo red and 1-anilinonaphthalene -8-sulfonic acid (ANS), Z15_EAK exhibited an absorbance indicative of fibril formation. Z15_EAK gel was showed to capture higher fraction of IgG than EAK16-II gel in vitro. Upon subcutaneous injection to C57BL/6 mice, IgG molecules formulated in Z15_EAK gel exhibited local retention for up to 8 days while IgG formulated in saline or EAK16-II gel receded from the site of injection at earlier time points. Specifically, at IgG dose of 3.25 mcg, by day 2, the mean fluorescent intensity of a dye-labelled IgG delivered in Z15_EAK gel was two times higher than the same IgG delivered in EAK16-II gel (66.0% ± 11.3 sem vs. 32.5% ±6.0 sem; n=16). By day 8, the difference was almost four times (3.4% ±1.0 vs. 0.98% ± 0.3). Thus the novel Z15_EAK peptide is a biomaterials platform on which the pharmacokinetics of antibody drugs can be optimized in vivo.

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. Current research is trending towards the use of human cells for therapeutic and toxicity testing. In this study, micromasses are prepared with hMSCs transduced with a lenti-viral Collagen II promoter-driven GFP reporter for non-invasive analysis of chondrogenesis. hMSC-based micromass cultures was prepared in a fluidically-enabled bioreactor and co-cultured with human umbilical vein endothelial cells (HUVECs) encapsulated in a photocrosslinkable hydrogel, generating an MSC/HUVEC organotypic culture model (OCM) of limb bud chondrogenesis. We challenged the MSC/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 MSC 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 both HUVEC cord formation and MSC chondrogenesis in MSC/HUVEC limb bud OCMs. This result is consistent with thalidomide-induced inhibition of angiogenesis in vivo and subsequent limb reduction in humans. Western blot revealed increased cereblon production in thalidomide-treated cultures, indicating an appropriate target tissue and adverse outcome pathway for thalidomide-induced changes in the culture. We conclude that the novel hMSC-HUVEC limb bud OCM represents a reproducible and controlled model to screen for potential limb teratogens.

Nerve-Specific Extracellular Matrix Hydrogel Enhances Recovery of a Peripheral Nerve Crush Injury

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Peripheral nerve injury commonly results in loss of neuromuscular function, often resulting in significant impact upon both quality of life and cost of care for patients. While crushed nerves represent a less severe injury, patients still face long recovery times and surgeons lack any procedure to aid patient recovery. One promising target for improving patient outcomes is the use of a peripheral nerve specific extracellular matrix hydrogel (PNECM) as an injectable, regenerative support. PNECM is an injectable gel generated from the extracellular matrix of healthy porcine nerve tissue. PNECM provides a tissue-specific microenvironment which is conducive to nerve repair, including: nerve specific growth factors that promote neurite outgrown, as well as factors that modulate the macrophage inflammatory response to injury. The PNECM was validated for removal of immunogenic cellular components, retention of beneficial nerve specific proteins, and tested for bioactivity in a series of in vitro and in vivo experiments. We found that PNECM significantly enhanced Schwann cell migration and axon extension in vitro. We also confirmed that exposure to PNECM generates a unique macrophage phenotype and a greater M2:M1 ratio than negative controls. In a rat model of sciatic nerve compression, the PNECM was injected directly into the injured nerve tissue. Animals were followed for 90 days while collecting functional metrics regularly. Compound motor action potential (CMAP) was also collected after 90 days. Both treated and untreated crush injuries recovered normal functional characteristics after 8 weeks but still showed a deficit in CMAP amplitudes at the end of 12 weeks. PNECM treated crush injuries showed a 50% increase CMAP amplitudes over untreated crush injury. As an easily injectable material which promotes recruitment of alternately activated, M2 macrophages and axon extension, we believe that PNS-ECM could significantly improve quality of life for affected patients.

Improved Adult and Pediatric Aortic Elastogenesis Driven by Adipose-Derived Mesenchymal Stem Cell Secreted Factors

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Introduction

Aortic aneurysms (AA) are enlargements of the ascending thoracic or infrarenal abdominal aorta that occur in aging populations, and pediatric patients with connective tissue disorders. Mediated by inflammatory damage to elastin, dilating AAs weaken and ultimately rupture or dissect. Currently, aortic diameter is the primary determining factor for surgical decisions. Adults AAs over 3cm, and pediatric AAs exceeding 0.5cm/yr growth rate typically undergo surgical intervention, often with repeated surgeries and broad-targeted therapies (beta blockers, ACE inhibitors). An elastin-targeted regenerative therapy could offer a non-surgical option for both patient groups. Our work has shown that periadventitial adipose-derived mesenchymal stem cell (MSC) delivery to a growing mouse aneurysm slows dilation and preserves elastic lamellae. This study tested whether MSC-secreted factors induce elastic fiber production by vascular smooth muscle cells (SMC).

Methods

Healthy adult human SMCs were purchased from ATCC. Pediatric SMCs were isolated from post-Ross procedure ascending thoracic aorta and aortic root tissue of a two-year old patient. Both cell types were loaded within 3D fibrin gel constructs in the presence or absence of human adult adipose-derived MSC secreted factors (SF). Elastin and elastin-chaperone mRNA transcription was measured using qPCR analysis. Elastic fiber morphology was analyzed by indirect immunofluorescence (and MATLAB-analyzed fiber microarchitecture). Insoluble elastin fragments were measured by ninhydrin assay.

Results & Discussion

After 20 days, while healthy adult SMCs produced insoluble elastin (1.79μg) without stimulation, SF stimulation induced a 152% increase in the percent of total protein that was insoluble elastin (0.22±0.15% control vs 0.56±0.37% SF). Imaging revealed continuous elastic fibers, with quantification revealing increased intersection density with SF. qPCR analysis suggested a SF-induced increase in expression of fibulin-5 and lysyl oxidase. Pediatric SMC SF-stimulation paralleled the results with adult cells, specifically showing a 226% increase in insoluble elastin deposition when compared to control (0.05±0.005% control, 0.19±0.05% SF).

Conclusion

MSC secreted factors are capable of stimulating elastic fiber assembly, through elastin and chaperone proteins, in both adult and pediatric SMCs. This could potentially lead to a non-surgical regenerative therapy for treatment of AA.

Graphene-based Biocompatible and Transparent Micro-Electrode Arrays for Simultaneous Electrical and Optical Measurements of Electrogenic Cells

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Investigating electrical activity of cardiomyocytes and neurons is essential to understanding their physiology, and mechanisms of cardiac and neurological diseases. Over the last few decades, a wide variety of bioelectrical interfaces, such as electroencephalogram (EEG), electrocorticography (ECoG), silicon-based electrodes (Utah arrays), metal based micro-electrode arrays (MEAs) and patch clamp have been developed. However, their performance is limited by factors such as poor biocompatibility of implant materials, mechanical mismatch of the devices with the tissue, high opacity or low spatial resolution. On the other hand, fluorescent indicators such as calcium sensitive dyes, provide very high spatial resolution, capable of resolving signals at single cell level, however, they are limited in volume measurements and temporal resolution. To overcome these limitations, we use graphene to fabricate MEAs due to its outstanding electrical conductivity (charge carrier mobility up to 200,000 cm2 V-1 s-1), mechanical flexibility, and high transparency (optical transmittance of ~97.7%). High electrical conductivity of graphene leads to recording of electrical signals at high signal-to-noise ratio, while its high flexibility minimizes the mechanical mismatch between the tissue and electrodes. Graphene's biocompatibility enables long term stable integration of cells with the devices. The high transparency of this material allows simultaneous electrical and optical studies, integrating the advantages of MEAs with fluorescent indicators, thus leading to high temporal and spatial resolution recordings. In addition, the transparency enables imaging modalities to monitor cellular morphology and intracellular markers that are indicative of cell health. 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.

Extracellular Matrix Hydrogel Downregulates Neoplastic Esophageal Cell Phenotype

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Tumor resection requires a suitable biomaterial that will not promote infection or re-occurrence of disease, and ideally reconstruct the tissue; but limited options exist. In 2011, our group treated 5 esophageal adenocarcinoma (EAC) patients (T1A) with a heterologous, xenogeneic extracellular matrix (ECM) bioscaffold after aggressive mucosal resection. The patients functionally preserved their esophagus, did not stricture, and most importantly, did not show reoccurrence of cancer to date. Since then, 9 more patients have been treated with similar results, and are 1-8 years disease-free. The described study is in a Phase I Clinical Trial, but before widespread clinical translation, an understanding of the molecular mechanisms is needed. The objective of the present study was to evaluate the effect of ECM bioscaffold degradation products (formed by pepsin-solubilization of the bioscaffolds) from non-malignant, decellularized tissue on normal (Het -1A), cancer pre-cursor (CP-A), and neoplastic (SK-GT-4, OE33) esophageal epithelial cells. ECM from heterologous urinary bladder matrix (UBM) and esophageal mucosa (eECM) were evaluated as candidate therapies. The two ECM tissue sources showed similar but distinctive effects on cell morphology, cell function (metabolism, proliferation, apoptosis), and EAC signaling pathways (gene, protein expression) compared to pepsin control. Both ECM tissue sources decreased OE33 cell proliferation and EAC signaling pathway PI3K-AKT gene expression at 24h, and decreased OE33 phosphorylated AKT as early as 6h for eECM and at 24h for UBM. The two tissue types also showed distinctive effects to downregulate neoplastic phenotype: UBM decreased OE33 and SK-GT-4 cell metabolism and increased CP-A cell apoptosis; while eECM decreased SK-GT-4, CP-A, and Het-1A cell proliferation; and showed robust downregulation of OE33 cell cycle/DNA replication signaling. The present study supports the continued investigation of ECM bioscaffolds to treat EAC patients in the Phase I clinical trial.

Soft Tissue and Drug Delivery System Development for Craniofacial Reconstruction after Injury

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Craniomaxillofacial trauma occurs in nearly half of battlefield injuries. In a 10-year period, craniomaxillofacial battle injuries to the head and neck were found in 42.2% of patients evacuated out of theater. Unfortunately, restoration of facial soft tissue defects remains complex. Fasciocutaneous flap transfer, the current standard of care, remains surgically challenging and risks failure. The objectives of this research are to address facial reconstruction with muscular and adipose therapies for restoring function and aesthetic, performing the following: (1) Identify and validate a biocompatible, resorbable technology capable of controlled volume elution capable of supporting adipose structure and volume retention. (2) Bioprint muscular structures capable of restoring function and power after injury. (3) Combine therapies in a single model, testing efficacy for recreation of complex facial depots after traumatic injury.

Optimization of Imaging Conditions and Analysis Methodology for Characterization of Properties and Quality Control of Biomimetic Poly(ester urethane) Urea Scaffolds, Silk Scaffolds and Collagen-Elastin Scaffolds for Vascular and Urethral Tissue Engineering

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Aim: Micro-porous scaffolds are used in many regenerative tissue engineering therapies. In this pilot study we preliminarily evaluated the use of microcomputed Tomography (microCT), used in conjunction with contrast-enhancement techniques, as applied to three types of bi-layered, biodegradable scaffolds designed for use in vascular and urethral regeneration. We furthermore explored the related use of analytical tools originally developed for bone 3D morphometry analysis.

Materials and Methods: Poly(ester urethane) urea (PEUU), silk based (lyophilized silk hydrogel and freeze dried silk solution) and collagen-elastin based scaffolds were imaged on a Scanco microCT 50 (Scanco Medical, Switzerland) scanner at a nominal resolution with the voxel size corresponded to 1/3-1/5 of the lowest pore size inside the scaffolds. The same was repeated after infiltration of the specimens with a 0.3% phosphotungstic acid solution as a contrast agent. The images produced were processed by the Scanco 3D Bone Morphometry software, with an output including a profile of the pore and strut sizes, connectivity density and pseudo-colored maps of pore and strut thicknesses.

Results-Conclusions: MicroCT imaging is an effective means for characterization of the properties and quality control of the constructs, through analysis of spatial distribution of the properties examined and identification of manufacturing-originated macro-defects and information within the constructs. 2D views of the parameters examined (pore size being the most informative) made it possible to visualize this spatial distribution and can be part of a standardized similar analysis package. The addition of a contrast agent was observed to enhance quantitative analysis precision for a standard nominal resolution, implying that development of related protocols for different strut materials or contrast agents (e.g. micro-filled resin) can contribute to optimization of a microCT-based analysis.

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A Role for Telomerase in Valvular Calcification

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Telomerase (TERT) is an enzyme best known for its telomere-extending actitivities on the ends of chromosomes, however, less known are the non-canonical, transcriptional and epigenetic actitivies of TERT. We sought to assess if TERT, through non-canonical activities, contributes to the progression of calcific aortic valve disease (CAVD). It has been established that overexpression of TERT in mesenchimal stem cells primes these cells to differentiate into osteoblasts. Other studies have identified non-canonical roles for TERT in inducing the transcription of genes in inflammatory and cell differentiation pathways. Our preliminary data shows that TERT is highly expressed in CAVD valves compared to Control valves. Under osteogenic differentiation conditions Control valve interstitial cells (VICs) upregulate TERT protein levels and calcify. Inflammatory signals induce TERT expression in VICs and exacerbates calcification. Similarly, wild type vascular smooth muscle cells (VSMCs) readily calcify in vitro, but VSMCs from TERT knockout mice do not, providing evidence that TERT is necessary is the osteogenic switch of a healthy to a calcifying VIC. Knocking down TERT reduces expression of the osteogenic transcription factor RUNX2 and we further provided evidence that STAT5 may help to mediate TERTs effects on osteogenic gene transcription. From these data we hypothesise that that TERT is required for valve calcification by inducing the osteogenic transition of quiescent valve interstitial cells (VICs) into calcifying VICs, and that TERT and STAT5 co-regulate transcription of osteogenic genes. These results indicate that TERT is an active contributor to the calcification process of valve tissues and our futures studies will delineate the mechanisms involved.

Nanoparticle Crosslinkers: Increasing the Efficacy of Surface Modification for Neural Implants

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Implantable neural electrodes provide a powerful tool for understanding the brain as well as therapeutic potential for functional restoration. However, implanted devices elicit an inflammatory immune response from the host, which results in glial encapsulation, neuronal degradation/migration from the implant, and a chronic decrease in recording quality and yield. Modifying the surface of electrodes with the neural adhesion protein L1 has been shown to reduce the glial activation, while also providing a substrate favorable for neural attachment. However, surface modification is limited by the low surface area of smooth substrates. Roughening the surface will allow for increased binding of L1, increasing the efficacy of the surface modification. Silica nanoparticles are well characterized as biocompatible and have tunable surface properties. In this work, we describe a silica nanoparticle linker between smooth substrates (silicon or glass) and L1, laminin, or the immobilizable antioxidant iSODm, increasing the roughness and surface area available for immobilization.

Biomaterials to Simultaneously Promote Angiogenesis and Lymphogenesis

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Biodegradable biomaterials exhibiting angiogenic and lymphogenic properties are expected to have applications in tissue engineering and wound healing. Even though numerous approaches have been investigated, achieving functional vascularization, including blood and lymph capillaries, remains a major challenge. Moreover, limited investigations have attempted to simultaneously stimulate functional blood and lymphatic networks in tissue engineered scaffolds. The embryonic chorion represents a highly vascularized membrane in mammals, birds and reptiles, and clinically approved human-sourced amnion/chorion allografts have been shown to be pro-angiogenic. As an alternative to human-sourced chorion, we have previously identified FGF2-mediated pro-angiogenic properties of porcine aortic adventitia (pAdv) extracellular matrix (ECM). As yet another alternative, here we hypothesized that decellularized chick chorioallantoic membrane (dCAM) retains bioactive signals capable of regenerating blood and lymph vasculature. Decellularized bioscaffolds made from CAM and pAdv had minimal DNA compared to respective native tissues, validating the decellularization process. Pepsin-digested lyophilized dCAM and pAdv powders formed hydrogels within 90 min at physiological pH, temperature, and rate similar to that of decellularized porcine small intestine submucosa (pSIS). dCAM and pAdv bioscaffolds increased proliferation of human and murine endothelial cells, directed endothelial cell migration, and promoted formation of tube-like structures in vitro, and they increased vascular invasion and capillary formation in the chick CAM assay. dCAM and pAdv digests also promoted invasion of Prox1+ lymphatic endothelial cells and stimulated formation of small and large diameter capillaries in a mouse subcutaneous plug assay. This study demonstrates that dCAM and pAdv hydrogels retain pro-angiogenic and pro-lymphogenic signals and supports their potential translation towards vascular regeneration in clinical applications.