



National Institute of Biomedical Imaging
and Bioengineering

2016 TRAINING GRANTEES MEETING ABSTRACT BOOK



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Biomedical Imaging
and Bioengineering

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1. Imaging characteristics of pediatric diffuse midline gliomas based on the presence of a poor prognostic marker histone H3 K27M mutation

Mariam S Aboian, David Solomon, Erin Felton, Sabine Mueller et al

University of California San Francisco

Purpose: The molecular basis underlying pediatric gliomas is distinct from adult gliomas. One unique molecular alteration that has been identified in pediatric gliomas is K27M missense mutation in histone H3 variants and presence of this mutation correlates with poor prognosis. The forthcoming 2016 WHO Classification will include “diffuse midline gliomas with histone H3 K27M mutation” as a new diagnostic entity. We identify imaging characteristics of these diffuse midline gliomas in pediatric patients based on the presence of histone H3 K27M mutation. **Materials and Methods:** We identified 42 pediatric patients (<20 yrs old) with diffuse gliomas with available MRI imaging. Histopathologic subtypes included diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma. We evaluated the imaging patterns of these diffuse gliomas based on their location, enhancement pattern, and necrosis. **Results:** In these 42 patients, tumors were supratentorial in origin in 48.8% of cases, infratentorial in 46.5%, and cervical spine in 4.7%. 74.4% of the tumors were midline (thalamus, pons, or spinal cord) with 71.9% of these had K27M mutation. All tumors in cerebral hemispheres were histone H3 WT and were associated with high amount of necrosis ($p < 0.003$). All cervical spine tumors were K27M mutant and had distal subependymal metastases within the lateral ventricles on follow up at 5-13 months. Majority of the infratentorial tumors were K27M mutant (83%), while only 67% of the thalamus tumors had K27M mutation. When comparing histone H3 mutant to WT midline gliomas, there was no statistically significant correlation between enhancement or border characteristics, infiltrative appearance, or presence of edema. **Conclusion:** Majority of diffuse midline gliomas originating in the thalamus, pons, or spinal cord were found to harbor histone H3 K27M mutation. Tumors arising in the cervical cord showed propensity for distal metastatic spread. In contrast, diffuse gliomas in the cerebral hemispheres were uniformly negative for K27M mutation and were more likely to demonstrate necrosis. **Clinical Relevance:** We describe the imaging features of a new WHO class of diffuse gliomas, Histone H3 K27M mutant gliomas.

2. Embolization of Arteries for the Treatment of Obesity (BEAT Obesity): 6 Month Safety and Efficacy Data

Olaguoke Akinwande, Clifford Weiss

Johns Hopkins School of Medicine

Purpose Bariatric Arterial Embolization (BAE) is an endovascular procedure targeting the gastric fundus for the treatment of obesity. BAE results in decreased serum ghrelin and weight modulation in animal models. BEAT Obesity study was initiated to assess the safety and efficacy of BAE to treat severely obese patients using 300-500 μ m Embospheres. Material and methods Adult otherwise-healthy, morbidly obese patients (BMI=40-60; n=7) were enrolled. Primary safety and efficacy endpoints were 30-day adverse events (AE) and weight loss. Secondary endpoints were serum obesity hormones (Ghrelin, Leptin, GLP-1, PYY), hunger/satiety assessments, Quality of Life (QOL) surveys, blood pressure, lipid profile, endoscopy and gastric emptying results. Six-month data, as collected to date, are presented. Results Mean age was 36.4 \pm 19.1 years, and BMI was 44.6 \pm 2.77 kg/m² at enrollment. Left gastric artery (LGA) embolization was performed in all patients. Additionally, the gastroepiploic arteries were embolized in 4 patients. There were 3 minor AEs with no major AEs. One patient had sub-clinical pancreatitis, 2 patients had a small (<1cm) superficial mucosal ulcers, one was healing by 2 weeks, and one was healed by 3 months. There was 5.9 \pm 2.4%, 9.5 \pm 3.1% and 13.3 \pm 4% excess weight loss at one, three and six months, respectively. Mean percent change of serum ghrelin from baseline was +8.68 \pm 34.74% at 1 month and -17.49 \pm 28.98% at 3 months. A trend towards improvement in QOL parameters was noted. Hunger/appetite scores were markedly decreased post-BAE and remained suppressed. Conclusion Early follow-up of BAE demonstrates safety, weight loss and associated appetite suppression in severely obese patients.

3. Convex Optimized Diffusion Encoding (CODE) gradient waveforms for bulk motion compensated cardiac Diffusion Weighted MRI

Eric Aliotta, Holden H Wu, Daniel B Ennis

UCLA

Purpose – To implement and evaluate Convex Optimized Diffusion Encoding (CODE) gradient waveforms for bulk motion compensated cardiac Diffusion Weighted MRI (cDWI) with minimum TEs. Introduction – cDWI has the potential to characterize cardiac microstructure without the need for a Gadolinium-based contrast agent (GBCA), which is important for the large number of patients with poor renal function requiring evaluation by cardiac MRI[1]. The clinical utility of cDWI, however, has been limited by severe sensitivity to cardiac motion. Recent reports of motion compensated (MOCO) diffusion encoding gradients with nulled first (M1) and second (M2) moments have demonstrated robustness to bulk cardiac motion[2, 3], but they necessarily increase the echo time (TE) compared to monopolar encoding (MONO). Increased TEs reduce SNR and limit spatial resolution. We have developed a MOCO cDWI sequence that employs Convex Optimized Diffusion Encoding (CODE) to reduce bulk motion sensitivity and shorten TE compared to existing MOCO schemes. Methods – Gradient Design: CODE diffusion encoding gradients were designed using convex optimization to determine the M1 and M2 nulled gradient waveform that minimizes TE for a target b-value while conforming to hardware constraints ($G_{Max}=74\text{mT/m}$ and $SR_{Max}=50\text{T/m/s}$) and pulse sequence timing. Healthy Volunteer Imaging: Healthy volunteers ($N=10$) were scanned on a 3.0T scanner (Siemens Prisma) after providing written informed consent. High resolution cDWI were acquired in the left ventricular (LV) short-axis with $b=350\text{s/mm}^2$, $1.5\times 1.5\times 5.0\text{mm}$ spatial resolution, 2x GRAPPA acceleration, three orthogonal diffusion encoding directions and three signal averages in a single 15-heartbeat breath hold. Both MONO ($TE/TR=67\text{ms}/1R-R$) and CODE-M1M2 encoding ($TE/TR=76\text{ms}/1R-R$) were acquired at eight subject-specific cardiac phases distributed across systole and diastole. Reconstruction and Data Analysis: Apparent diffusion coefficient (ADC) maps were reconstructed for each cardiac phase. Motion corrupted voxels were identified by ADC values exceeding $3.0\times 10^{-3}\text{mm}^2/\text{s}$ (the diffusivity of free water at 37°C , a thermodynamic upper bound for soft tissues) in the LV. The mean myocardial LV ADC and the percentage of motion corrupted LV voxels were then calculated for each phase. Statistical analyses were performed using t-tests with Holm-Sidak post hoc corrections. Clinical Imaging: Patients ($N=5$) undergoing routine clinical cardiac MRI exams were also scanned after providing written informed consent. cDWI were acquired before and after the injection of a GBCA (Gadovist, Bayer Healthcare) using the CODE-M1M2 cDWI protocol at a single early systolic phase (100ms delay from the QRS complex via ECG). Mean myocardial LV ADC was calculated after manual segmentation for each patient. Results – The TE for CODE-M1M2 ($TE=76\text{ms}$) was 19% shorter than modified bipolar MOCO[3] ($TE=94\text{ms}$) for $1.5\times 1.5\text{mm}$ in-plane resolution and $b=350\text{s/mm}^2$ (Figure 1). With MONO the mean ADC values were significantly corrupted ($>3.0\times 10^{-3}\text{mm}^2/\text{s}$, $p<0.004$) for 50% of the cardiac phases whereas 0% of the cardiac phases were corrupted with CODE-M1M2 ($p=N.S.$) (Fig. 2B). CODE-M1M2 measured significantly lower mean ADCs than MONO ($1.9\pm 0.3\times 10^{-3}\text{mm}^2/\text{s}$ vs. $3.8\pm 0.6\times 10^{-3}\text{mm}^2/\text{s}$, $p<0.007$) and

fewer motion corrupted voxels ($14\pm 14\%$ vs $67\pm 21\%$, $p < 0.0006$) in 100% of the cardiac phases (Fig. 2C). The clinical CODE-M1M2 cDWI scans were largely free of bulk motion artifacts (Fig. 3) and ADC maps were in agreement with myocardial diffusivities measured in volunteers. There was no significant difference between mean ADCs measured pre- and post-contrast (mean $ADC_{Pre} = 1.46 \pm 0.2 \times 10^{-3} \text{mm}^2/\text{s}$, $ADC_{Post} = 1.58 \pm 0.3 \times 10^{-3} \text{mm}^2/\text{s}$, $P = \text{N.S.}$). Discussion – The volunteer study demonstrated that cDWI with CODE-M1M2 mitigated bulk motion artifacts and substantially increased the range of cardiac phases that can accommodate robust ADC measurement. While previous approaches which have required careful selection of the sequence timing and several repeated acquisitions[4], CODE-M1M2 was successful for all patients imaged using a single, predetermined 100ms ECG delay. These findings echo previous reports[3, 5, 6] of cDWI with M1M2 nulled encoding. Myocardial ADC values (1.4 to $1.6 \times 10^{-3} \text{mm}^2/\text{s}$) were also in agreement with these reports. The agreement in ADC values between pre- and post-contrast imaging highlights the relatively weak T1 dependence of the sequence for characterizing myocardial microstructure, which is important for the interpretation of ADC values in patients that may not receive contrast. Conclusion – CODE-M1M2 cDWI significantly improved robustness to cardiac bulk motion compared to MONO cDWI. CODE-M1M2 cDWI also permits first and second moment nulling with a shorter TE than existing MOCO cDWI methods. References: 1. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382(9889):339-52. 2. Nguyen C, Fan Z, Sharif B, He Y, Dharmakumar R, Berman DS, et al. In vivo three-dimensional high resolution cardiac diffusion-weighted MRI: A motion compensated diffusion-prepared balanced steady-state free precession approach. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2013;72(5):1257-67. 3. Stoeck CT, von Deuster C, Genet M, Atkinson D, Kozerke S. Second-order motion-compensated spin echo diffusion tensor imaging of the human heart. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2015. 4. Rapacchi S, Wen H, Viallon M, Grenier D, Kellman P, Croisille P, et al. Low b-value diffusion-weighted cardiac magnetic resonance imaging: initial results in humans using an optimal time-window imaging approach. *Investigative radiology*. 2011;46(12):751-8. 5. Nguyen C, Fan Z, Sharif B, He Y, Dharmakumar R, Berman DS, et al. In vivo three-dimensional high resolution cardiac diffusion-weighted MRI: A motion compensated diffusion-prepared balanced steady-state free precession approach. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2013. 6. Welsh C, Di Bella E, Hsu E. Higher-Order Motion-Compensation for In Vivo Cardiac Diffusion Tensor Imaging in Rats. *IEEE transactions on medical imaging*. 2015.

4. Assessment of Gd-doped Silica Microshells for Prostate Ultrasound HIFU Therapy Sensitization and Targeted Drug Delivery

Gregory Anthony, James Wang, Andrew Kummel, Ph.D., Steffen Sammet, M.D., Ph.D.

University of Chicago

Purpose: To assess the contrast properties and relaxivity of silica microshells with gadolinium oxide (Gd₂O₃) incorporated into the shell surface or encapsulated in the interior. This study also aims to optimize agarose phantom fabrication for in vitro imaging of the microshells. Finally, we demonstrate the capabilities of our prostate HIFU transducer for single- and multi-element heating. **Methods:** Test tube phantoms with varying type and consistency of agarose were created and studied. 2% agarose test tube phantoms were created with varying concentrations of two types of silica microshells: intact shells with Gd₂O₃ incorporated into the surface only, and ruptured shells which initially contained Gd₂O₃ in the interior only. Test tube agarose phantoms with varying concentrations of gadodiamide (Omniscan) were also created. T1-mapping was performed via inversion recovery SE sequences with varying TI and curve-fitting on a voxel-by-voxel basis. T2-mapping was performed using multi-echo SE sequences with varying TE and curve-fitting on an ROI basis. T1 and T2 relaxivities (r1 and r2) were obtained as the slope of 1/ΔT1 and 1/ΔT2, respectively, versus Gd concentration in the samples. Proton-resonance frequency (PRF)-based MR thermography images were obtained upon sonication of a cylindrical agarose-silica phantom with a transurethral prostate HIFU transducer. All images were acquired on a Philips Achieva dStream 3T MR scanner (Philips Healthcare, Netherlands). **Results:** 2% by weight, non-low-electroendosmosis agarose was selected for phantom fabrication to balance gel strength with minimal T2 reduction. Compared with equal molar concentrations of gadolinium in gadodiamide, surface-doped microshells showed reduced signal enhancement in T1-weighted images, while ruptured microshells with encapsulated Gd₂O₃ showed little enhancement. Intact, surface-doped shells had an r1 and r2 of 1.17 and 37.5 L/mmol-s, respectively. Ruptured, Gd-encapsulating shells had an r1 and r2 of 0.806 and 177 L/mmol-s, respectively. Gadodiamide gel tubes showed an r1 and r2 of 3.11 and 13.9 L/mmol-s, respectively. MR thermography images showed uniform heating from the central four elements of the prostate transducer. When heating with a single element at 4 W for five minutes, the width of the thermal dose ≥ 240 EM (43°C) region was measured at 6.4 mm along the transducer and 6.1 mm perpendicular to the transducer. **Conclusion:** An effective agarose phantom for imaging Gd-doped silica microshells has been developed. Surface-doped microshells can be easily visualized at Gd concentrations of 0.3 mmol/L or higher. Large agarose-silica phantoms are also suitable for imaging heating patterns of the prostate transducer.

5. Radiomics of breast cancer: A robustness study

Natalia Antropova, Hui Li, Karen Drukker, Li Lan et al

University of Chicago

Purpose: Computer-extracted image phenotypes (CEIPs)/features are being investigated as complimentary attributes in the characterization of breast cancer in radiomics/ radiogenomics research. To be clinically useful, CIEPs need to be robust across data obtained with MRI scanners of different manufacturers and imaging protocols. Methods: Our research involved two HIPAA-compliant retrospectively-collected MRI datasets: Database 1 included 91 imaged breast cancers from the National Cancer Institute repository (imaged using General Electric equipment) and Database 2 included 332 breast cancers (imaged at our site using Phillips equipment). For each case, information on clinical lymph node (LN) status and histopathology on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) were available. Each lesion underwent quantitative radiomics analysis yielding CEIPs characterizing tumor size, shape, morphology, enhancement texture, kinetic curve assessment, and enhancement variance kinetics. The robustness evaluation involved comparing feature values and feature performance in the task of distinguishing LN, ER, PR, and HER2 status between two datasets. Prior to the evaluation, datasets were matched in terms of pathology and tumor size. T-test was used to compare average feature values for the entire datasets and for clinical subgroups of interest. Superiority testing was used to evaluate the differences in feature performance in the prognostic tasks, with area under the receiver operating characteristic curve (AUC) serving as a figure of merit. The features that failed to show statistically significant differences in performance were further evaluated with non-inferiority testing. Results: We failed to find any statistically significant differences in the average value of the features from tumor size category. Greater variation in average feature values for the clinical subgroups having less than 20 cases. In the prognostic tasks, features showed varying levels of robustness. The best agreement in performance was seen in the lymph node classification for two features -- tumor morphology and tumor heterogeneity -- with absolute value of the lower bound of the 90% confidence interval for $\Delta AUC < 0.05$. Conclusion: Quantitative breast radiomic features show varying robustness in their average values and in performance across MRI scanners. Non-inferiority testing revealed radiomic features with robust performance in the classification tasks. In practice, features showing different performance need to be tuned based on the MRI scanner used during imaging procedure.

6. Targeted Nano-delivery of Immunosuppressive Agents in Transplantation

Baharak Bahmani, Mayuko Uehara, Qiaobing Xu, Reza Abdi

Harvard Medical School

Purpose: Although discovery and development of immunosuppressive agents (ISA) have improved the allograft and recipient survival rate in organ transplantation, there are drawbacks associated with administration of ISA that has limited their success in eliminating acute and chronic rejection. Other side effects of ISA are post-transplant malignancies as well as accelerated cardiovascular disease. Innovative drug delivery approaches are required for efficacious delivery of ISA to overcome their severe side effects. Nanoparticle-based drug delivery is an exciting platform with tremendous potential and advantages including controlled release, targeted delivery and reduced toxicity. By enveloping ISA in a biodegradable nanoparticle, significantly lower systemic dosage of ISA is required resulting in reduced toxicity, enhanced bioavailability and efficacy of the drug. We have designed nanoparticles to deliver ISA to the lymph nodes and the allograft to reduce possibility of graft rejection. The targeting moiety used in this study is monoclonal antibody MECA-79, which collectively binds to the peripheral node addressin (PNAd) molecules expressed in high endothelial venules (HEV). **Method:** To study trafficking of nanoparticles to lymph nodes, we fabricated poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with a near infrared fluorescent dye, IR 800CW. The nanoparticles were synthesized using a single step self-assembly method called nanoprecipitation/solvent evaporation. Briefly, mixture of PLGA and IR 800CW in the organic solvent was added drop wise to deionized water under vigorous stirring and was stirred to evaporate the organic solvent. Nanoparticles were recovered and washed by centrifugation. We have used maleimide-PEG-amine to modify the nanoparticles surface and conjugate MECA-79 antibody to the surface of the nanoparticles. To study distribution kinetics of particles in vivo and examine nanoparticles internalization and retention at the level of HEV in real time, we administered MECA-79 conjugated IR 800-loaded nanoparticles intravenously to the mouse with and without rejected allogeneic skin transplant. **Results and Conclusion:** The in vivo live fluorescence imaging on mouse using iBox Explorer2 imaging microscope 24 hours post IV administration of nanoparticles suggests that MECA-79-conjugated nanoparticles accumulate at a higher level in the lymph nodes as compared to nanoparticles without MECA-79 antibody (control sample). Our preliminary results provide strong evidence that MECA-79 conjugated nanoparticles traffic much more efficiently to the draining lymph node in the transplanted animals compared to non-draining lymph nodes. We have demonstrated successful conjugation of nanoparticles with monoclonal antibodies against PNAd in HEV and targeted delivery to the lymph nodes. These nanoparticles present a promising nano-platform for targeted delivery of ISA to the lymph nodes.

7. High Frequency QRS Analysis (HF-QRS) has Incremental Diagnostic Accuracy over ST-Segment Analysis Alone for the Detection of Myocardial Ischemia

Pelbreton Balfour Jr. MD ScM, Jorge Gonzalez, MD, Peter Shaw MD, Margarita Pérez MD et al
University of Virginia Health System

Purpose: High-frequency QRS analysis (HF-QRS) is a novel tool to supplement standard ST-analysis during stress ECG. We sought to compare the diagnostic accuracy compared with standard ST-analysis for the identification of any and significant myocardial ischemia by exercise SPECT myocardial perfusion imaging (MPI). **Methods:** We analyzed the diagnostic accuracy of HF-QRS versus ST-depression analysis in 257 consecutive patients who underwent exercise stress SPECT MPI. An ischemic HF-QRS pattern was defined as an absolute reduction of ≥ 1 μV and a relative reduction of $\geq 50\%$ between maximal and minimal values of the mean root square of the 150-250 Hz band signal in ≥ 3 leads. Semi-quantitative gated SPECT MPI was the gold standard, with $\geq 10\%$ of the LV considered significant ischemia. Statistical analysis was performed using chi-square and logistic regression analysis. **Results:** The study cohort was 67% male with a mean age of 59 ± 12 . Myocardial ischemia was present in 45 patients (17.5%) and significant ischemia ($\geq 10\%$ of the left ventricular (LV) myocardium) in 13 patients (5.1%). ST segment and HF-QRS analyses were positive in 9 (3.5%) and 57 patients (22.2%), respectively. HF-QRS had substantially higher sensitivities than ST-analysis for any (60.0% vs. 20%, $p < 0.01$) and significant (84.6% vs. 38.5%, $p < 0.01$) ischemia while maintaining similar specificities ($p=0.90$ for any ischemia). HF-QRS demonstrated incremental diagnostic value to ST-analysis and clinical risk factors and increased the model discrimination (AUC 0.74 vs 0.80, $p < 0.0001$). **Conclusions:** A strategy of exercise stress ECG with HF-QRS analysis can identify significant ischemia with high diagnostic accuracy. This novel adjunct non-invasive method may improve CAD risk stratification in low-intermediate risk subjects.

8. Mixed Antigen/Adjuvant Peptide Amphiphile Micelles Improve Group A Streptococcal Vaccination

John Barrett, Joel Collier, Matthew Tirrell

University of Chicago

Streptococcus pyogenes (Group A *Streptococcus*, GAS) is a Gram-positive bacterium restricted to natural growth in humans. GAS frequently elicits diseases that range in severity from mild infections of the pharyngeal mucosa and dermis to life-threatening invasive infections of connective and muscle tissues leading to necrotizing fasciitis, impetigo, and toxic shock. Additionally, post-infection sequelae diseases such as acute rheumatic fever and glomerulonephritis arise following localized infections of the nasopharynx and skin, respectively. Epidemiological studies estimate that each year greater than 500,000 worldwide deaths are attributable to GAS infections, placing it among the top ten leading causes of death from infectious pathogens. In the United States alone, more than \$600 million is spent annually treating diseases caused by this organism with no effective preventative method established short of prophylactic antibiotic usage. Vaccines against GAS remain unavailable despite decade's worth of research and development. While whole-killed and live-attenuated vaccines have been tremendously effective in preventing pathogenic infections, they are also associated with undesirable side effects. Subunit vaccines that deliver just the peptide antigen of interest have been shown capable of stimulating an immune response. But these peptide antigens are generally weak immunogens on their own and require strong adjuvants (non-specific immunostimulants) to be effective. In order to enhance the immunogenicity of peptide vaccines, new delivery systems must be designed. Peptide amphiphiles are unique biomaterials comprised of peptide-lipid conjugates that undergo self-assembly into micelles in water and have been shown capable of delivering biologically active peptides for a variety of applications. Therefore, peptide amphiphile micelles provide a novel platform to improve the host immune response to peptide vaccines. The J8 peptide is a 29 amino acid, conformationally dependent B cell epitope that has been shown to generate an opsonophagocytic, high titer antibody response in mice. J8 was covalently tethered to a di-palmitic acid tail (J8-diC16) and fabricated into peptide amphiphile micelles in PBS. When delivered to mice subcutaneously, J8-diC16 was found to induce J8-specific high antibody titers greater than soluble J8 delivered with commercially available adjuvants. To further enhance the antibody response, mixed micelles comprised of J8-diC16 and amphiphilic adjuvants were synthesized. Mixed micelles induced a strong immune response after a single vaccination and higher titers than all other formulations. It was also discovered that micelles are capable of activating dendritic cells. The research presented in this poster demonstrates the promise peptide amphiphile micelles have in improving the field of vaccine engineering.

9. Platforms for Scattering Angle Resolved Optical Coherence Tomography Based Retinal Alzheimer's Diagnosis

Vikram L. Baruah, Michael R. Gardner, John Rector, Robert P. Striet et al

University of Texas at Austin

PURPOSE: Studies have recently shown a primary retinal pathology in neurodegenerative diseases. Increasing evidence suggests mitochondria tip towards a fission state in failing synapses, a precursor to the development of amyloid-beta ($A\beta$) plaques and neurofibrillary tangles, the classic histopathological indications of Alzheimer's disease. In accordance with Mie light scattering theory, mitochondria in fission are hypothesized to backscatter light at greater angles. Although sub-cellular changes in the retina are beyond the resolution of standard OCT, scattering angle resolved OCT (SAR-OCT) detects variations in backscattering angle not accessible in current retinal imaging approaches. In this study, cellular and tissue platforms were developed to support SAR-OCT Alzheimer's diagnostic development. **METHODS:** To investigate the general feasibility of deducing mitochondrial fission with SAR-OCT, fibroblasts were used as a living optical phantom. Fibroblasts were chosen due to their hardy nature and fast growth rate enabling rapid experimentation. Cells were repeatedly seeded to generate multilayer cultures resolvable by OCT. S-nitrosocysteine was used as a NO donor to induce mitochondrial fission. Tools for retinal flatmount imaging were developed to support more clinically relevant analysis. A novel retinal extraction method was used where the ora-serrata is the incision point, optical nerve is cut at the dorsal surface of the eye before extraction and the sclera is peeled leaving the retina adhering to the lens. Subsequently, the retina is placed in a novel temperature controlled tissue chamber. The chamber was designed with Caddock MP825 resistors to supply heat, thermoconductive polycarbonate to dissipate heat, thermocouples and PID controller to stabilize temperature of fluid bathing the retina, gravity fed inflow of solution and vacuum controlled outflow. SAR-OCT imaging was correlated to fission state by two photon imaging mitochondria in both multilayer fibroblast cultures and retinal flatmounts. **RESULTS:** Repeated seeding generated up to 50 μm thick primary cell cultures. Concerning tissue platforms, the retinal surgery technique enabled extraction of flatmounts with intact optical discs, visible through OCT. The tissue chamber maintained solution temperature at $33 \pm 0.72^\circ\text{C}$. Optical distortions due to temperature fluctuations and turbulent flow were imperceptible. **CONCLUSION:** The primary cell culture methods described provide a 3-dimensional optical phantom, without artifacts that may exist in hydrogel cultures. The novel surgical retinal extraction method and tissue chamber design provide highly intact retinal flat mounts and stabilized imaging. These methods serve as a platform supporting experiments involving SAR-OCT imaging of retinal mitochondrial fission, ultimately to establish non-invasive and early detection of Alzheimer's disease.

10. Removal of Targeted Pathways on Blood-Derived and not Brain-Derived Immune Cells Improves Intracortical Recordings

Hillary W. Bedell, MS, Madhumitha Ravikumar, PhD, Shushen Lin, Ashley Rein et al

Case Western Reserve

Action potentials from individual neurons can be recorded from intracortical microelectrodes affording these devices much potential in basic research and rehabilitation applications. Unfortunately, the quality of the neural signal decreases over time. Neuroinflammatory mechanisms play a major role in intracortical microelectrode failure. Two of the biological pathways that contribute to the failure of these devices are the breakdown of the blood brain barrier with subsequent myeloid cell infiltration and the Cluster of Differentiation 14 (CD14) pathway. CD14 is a key co receptor involved in the recognition of extravasated serum proteins and cellular damage in the brain resulting from intracortical microelectrode implantation. This work aims to delineate the role of the CD14 pathway on infiltrating macrophages versus resident microglia. Bone marrow chimera mice were used to selectively inhibit CD14 from either resident microglia or infiltrating myeloid cells. Intracortical microelectrodes were implanted into wild type (WT) C57/BL6, CD14 knock out mice (Cd14^{-/-}), and bone marrow chimera mice (Cd14^{-/+} WT bone marrow and WT + Cd14^{-/-} bone marrow). To evaluate the long-term stability of intracortical microelectrode performance, Cd14^{-/-}, bone marrow chimera mice (Cd14^{-/-} + WT bone marrow and WT + Cd14^{-/-} bone marrow) and WT control mice were implanted with functional intracortical Michigan style microelectrodes in the forelimb-associated motor cortex. Electrophysiological recordings were obtained twice a week to assess function of the electrode. The percent of channels of the electrode that are recording single units and number of single units per channel were tracked over time for 16 weeks post implant as metrics of recording quality. Two weeks post implantation, microglia/macrophage activation, astrocytic encapsulation, blood-brain barrier disruption, and neuronal dieback was assessed via immunohistochemistry. After two weeks, inhibiting CD14 on myeloid cells and not resident microglia reduced blood-brain barrier permeability and increased neuroprotection. Up to 10 weeks, the four conditions demonstrated comparable percent of channels recording single units for a given day. However, about 10 weeks after implant, the percent of channels recording single units for WT mice significantly decreases compared to Cd14^{-/-} and the two chimeric conditions. Interestingly, the percent of channels recording single units does not significantly decrease over time for the WT + Cd14^{-/-} BM mice. Our study identifies a clear link between specific inflammatory/immunity pathways and the long-term performance of intracortical microelectrodes. Further, our results suggest that systemic administration of therapeutic agents to inhibit the CD14 receptor-mediated pathway from blood-derived cells can be sufficient to improve chronic intracortical electrode performance.

11. Targetable, Complement-Modulating Janus Microparticles for Pathogen Clearance

Michael C. Bellavia, Brandon A. Holt, Todd Sulchek

The Georgia Institute of Technology

Purpose: Multidrug resistance in bacteria is a pernicious healthcare problem – methicillin-resistant *Staphylococcus aureus* (MRSA) is well-known, but certain Gram-negative (outer cell membrane, high surface lipopolysaccharide) strains, such as *Klebsiella pneumoniae*, resist almost all known antibiotics [1]. Herein we present a multifunctional biomaterial for targeted complement activation towards antibiotic resistant bacteria. Gold/polystyrene Janus microparticles with differentially-functionalized hemispheres were designed to target *Escherichia coli*, a model Gram-negative bacterium, and simultaneously activate cytotoxic processes via the complement system. The complement system is an integral component of the innate immune response, and describes a cascade of interacting proteins that opsonize and lyse foreign pathogens. This platform could allow for facile conjugation of a targeting moiety, such as an antibody specific to *E. coli* lipopolysaccharide (LPS), to better localize complement to the bacteria. **Materials and Methods:** Gold was deposited on a single hemisphere of commercially-available carboxylated polystyrene microparticles, and the two hemispheres were subsequently functionalized similarly to a previously reported scheme [2]. Gold presence on a single hemisphere was verified via energy-dispersive X-ray spectroscopy (EDS) and the proper conjugation scheme was observed with EDS and confocal microscopy. The gold hemisphere received a thiol-polyethylene glycol (PEG)-biotin conjugate in lieu of streptavidinylated anti-LPS antibody. Anti-bovine serum albumin (BSA) IgG was adsorbed randomly (non-oriented) or bound to a BSA coating (oriented) on the polystyrene hemisphere. The particles were then incubated with DH5 α *E. coli* and normal human serum at ratios of 10:1, 1:1 and 1:10 microparticle-to-cell number. Next, each *E. coli* and serum solution was diluted serially tenfold up to 1:10000 and plated in agar to evaluate the impedence of bacterial growth. All plates were incubated for 16 hr at 37°C and any colonies formed were counted manually. **Results:** The Janus particles with non-oriented antibody improve upon the cytotoxicity of the serum, in a manner directly proportional to serum concentration. The oriented antibody particles demonstrate less cytotoxicity, with unmodified polystyrene base particles demonstrating minimal cytotoxicity only at the highest microparticle ratio. These results suggest the role of antibody orientation in particle-mediated complement classical pathway activation. **Conclusions:** We have demonstrated via a particle platform that the orientation of the antibody displayed affects the cytotoxic capability of serum complement. As the gold hemisphere can be further modified with a targeting moiety, the diversity of the target pathogen is as extensive as the available antibody library. Future work will investigate the capability of the particles to localize complement to a particular pathogen strain. 1. Magiorakos, A.P., et al., *Clinical Microbiology and Infection*, 2012. 18(3): p. 268-281. 2. Tang, J.L., et al., *Langmuir*, 2012. 28(26): p. 10033-10039

12. Using Near Infrared Imaging to Assess Lymphatic Function in a Rat Osteoarthritis Model

Fabrice C. Bernard, Thanh N. Doan, J. Brandon Dixon, Nick J. Willett

Georgia Institute of Technology

Purpose: Osteoarthritis (OA) is disease of the joint that leads to degradation of the cartilage, joint destabilization, and eventually joint failure. The homeostasis of synovial fluid, which lubricates the joint, is maintained by the presence of blood and lymphatic capillaries in the synovial membrane. Inflammation is present in OA well before the development of significant radiographic changes. These inflammatory signals may potentially dysregulate lymphatic function and lead to the further progression of OA, as lymphatic clearance requires the active contractility of the collecting lymphatic vessels. The selective clearance of large molecules through the lymphatic system allows us to use near infrared (NIR) tracer attached to 20kDa PEG in order to image the knee space and assess functional lymphatic clearance from the joint as well as determine the functionality of the downstream lymphatic collecting vessels that drain the joint. **Methods:** Two preliminary experiments were performed. For joint clearance studies Lewis rats underwent either a sham surgery where only the MCL was transected or a medial meniscal transection (MMT) surgery where the MCL and the meniscus were transected. After surgery rats were injected intra-articularly with 20kDa PEG LICOR NIR dye and the joint was imaged at a fixed interval. The data was then fitted to an exponential curve and the rate of decay was determined from the curve fit. For functional collector vessel testing non-operated rats were injected subcutaneously near the knee joint in order to visualize and image collecting vessels. **Results:** Our preliminary findings suggest that there may be altered fluid drainage from the joint as a result of OA. This was shown by a decrease in intra-articular dye clearance in MMT animals up to 30 days after surgery. After 81 days the MMT and sham animals no significant difference in clearance was observed. We also demonstrated the ability to repeatedly image the lymphatics that drain the knee space to capture growth of lymphatic collaterals in response to surgery and quantify collecting vessel pump function. **Conclusions:** These experiments have given our group reliable modalities to quantify lymphatic function in the knee space in addition to downstream lymphatic vessels.

13. Probabilistic tractography of the corticospinal tracts using constrained spherical deconvolution more completely delineates motor pathways in children with cerebral palsy

Adam S. Bernstein, Theodore Trouard

University of Arizona

Purpose: In the last 10 years, huge advances have been made in the field of diffusion magnetic resonance imaging (dMRI). Image acquisition time continues to decrease, and more advanced algorithms for estimating the microstructural environment of tissues are becoming more practical for clinical imaging. However, the majority of clinical studies continue to employ the somewhat simplistic diffusion tensor model for both analysis of microstructure and the anatomy of neural pathways. The present work presents a preliminary result of using a more complete dMRI collection and processing scheme for applications in children with Cerebral Palsy. **Methods:** One 2 year old, female subject, diagnosed with cerebral palsy, was scanned pre (immediately) and post (36 weeks) physical therapy. The MRI protocol included a 64-direction, $b = 1000 \text{ s/mm}^2$ single shot EPI diffusion MRI sequence with 6 $b = 0 \text{ s/mm}^2$ volumes interspersed for motion correction. The resolution of the dMRI is 2.25 mm isotropic, with a TR/TE of 8150/109 ms. One $b = 0 \text{ s/mm}^2$ was collected with a reversed phase encode direction for EPI distortion correction. A T1-weighted image was collected for anatomical reference with 0.9mm isotropic resolution. Diffusion-MRI data was processed using FSL's TOPUP and EDDY for distortion correction, LPCA denoising was performed using MATLAB 2015b, and constrained spherical deconvolution, diffusion tensor fitting and probabilistic tractography were performed using MRtrix. **Results:** Tractography results from seed points in the cortical spinal tracts in the pons demonstrate increased coverage of the whole primary motor cortex, despite the large periventricular lesion, when using constrained spherical deconvolution based tractography relative to the tractography results based on the diffusion tensor model. Tracts that intersect the lesion using tractography based on constrained spherical deconvolution either terminate, or circumvent the lesion. Tractography performed using the diffusion tensor model shows only superior-inferior tracts with very little lateral coverage of the primary motor cortex. Further, no tracts that intersect the lesion make it to the cortex. **Conclusion:** Tractography based on a more robust interpretation of dMRI data more completely maps out pathways in the brain, and thus correlates better with the phenotype of the extent of motor dysfunction. The tractogram produced by the diffusion tensor model suggests that there are no connections with the motor cortex downstream of the lesion or at any of the more lateral regions of the motor strip. If this were truly the case, the affected children would be expected to demonstrate much more severe motor disability than they do. While it has been shown that most whole-brain tractography algorithms find many false-positive tracts, the more complete coverage of the motor strip produced by constrained spherical deconvolution (CSD) based tractography suggests that there is hope for dMRI to be used as a non-invasive diagnostic and prognostic test for the evaluation of cerebral palsy.

14. Tissue Microenvironment Training Program

Rohit Bhargava,

University of Illinois at Urbana-Champaign

A quantitative understanding of the tissue microenvironment (TiMe) is now recognized as critical for advancing biomedical science and healthcare, ranging from regenerative medicine to managing the burden of cancer. To this end, the integration of three technological approaches is essential: (a) sensing and imaging to measure biochemical and biophysical parameters, (b) bioengineering to recapitulate the TiMe, and (c) computational modeling to develop the next generation of productive scientific leaders. Here we describe a training program in which predoctoral students will integrate the use of the three technological approaches in TiMe-related studies using the biological contexts of disease and development. With intensive mentoring and systematic activities focused on professional development, trainees will become the next generation of interdisciplinary leaders capable of undertaking fundamental research and enabling translational advances. The University of Illinois at Urbana-Champaign has strong disciplinary programs, diverse faculty spanning the intellectual arc of the program, and appropriate facilities and unique resources in each of these technological areas. The program is outlined and key activities will be highlighted.

15. Developing Single Molecule Sensitive Fluorescent Tools for Studying RNA-Protein and Protein-Protein Interactions in Cells and Tissue

Emmeline Blanchard, Dr. Rachel Fearn, Dr. Philip Santangelo

Georgia Institute of Technology

An important tool in exploring intracellular events is a method of quantitatively looking at single molecule interactions in a cellular context. Evaluation of what protein-protein or protein-RNA interactions exist provides vital information about events occurring in a cell. Common techniques for deciphering cellular interactions, such as co-immunoprecipitation for protein-protein or RNA immunoprecipitation for protein-RNA are informative, but require measurement outside the cellular environment, so lose localization information and have the potential for altered interactions due to the buffers required. This research proposes to use and develop methods of detection for these interactions in a cellular environment. These methods could be used for numerous applications, such as exploring proteins involved in respiratory syncytial virus (RSV) replication or quantitatively detecting abnormal protein-RNA interactions in colon cancer samples. For protein-protein interactions, proximity ligation assays (PLA) were used. Additionally, modified RSV was created by Dr. Rachel Fearn with a HA tag on the M2-1 protein to allow for better PLA of M2-1 with different viral proteins. To detect RNA, fluorescent multiply-labeled tetravalent RNA imaging probes (MTRIPs) were created with sequences specific to the RNA of interest and used as fluorescent in situ hybridization (FISH) probes. PLA was then performed off an epitope tag on the MTRIP to investigate protein-RNA interactions. Using these tools, PLA was successfully able to be performed between the modified M2-1 and other viral proteins. It was found that at 6 hpi, the nucleocapsid (N), matrix (M) and phospho (P) proteins all interacted with the M2-1 protein in the cell. N and M, however, had visibly more interactions with M2-1 than P. Presence of viral mRNAs through the use of FISH at 6 hpi also indicated replication is already occurring at this early time point. Additionally through the use of MTRIPs and FISH, interaction assays between RNA and proteins were able to be performed. This was done with MTRIPs specific for the poly-A tail and the RNA binding protein, HuR, which is abnormally localized in colon cancer. Together, these tools provide critical information about interactions in a cellular environment. By developing and optimizing these, disease molecular interactions in a cellular context can be explored, gaining more accurate information to develop better detection methods and treatments.

16. Adipose-derived Mesenchymal Stem Cells Stimulate Elastin Production by Adult Human Smooth Muscle Cells in a 3D Fibrin Scaffold

Kory J. Blose, Justin S. Weinbaum, David A. Vorp

University of Pittsburgh

Adult human vascular smooth muscle cells (SMCs) do not normally produce elastin in-vivo, but do so in-vitro with treated with transforming growth factor beta-1 (TGF- β 1). As human adipose-derived mesenchymal stem cells (hADMSCs) are known to produce TGF- β 1, we hypothesized that adult SMCs co-cultured with hADMSCs would produce elastin. Constructs were made by embedding 6×10^4 commercially-sourced SMCs in fibrin gels. After two days, 9×10^5 commercially-sourced hADMSCs embedded in fibrin gels were added on top of the constructs. Additional experimental constructs were made without hADMSCs and treated with hADMSC conditioned media. Positive and negative control constructs lacking hADMSCs were treated with or without TGF- β 1, respectively. After 28 days of culture, constructs were imaged with an Olympus multiphoton microscope to visualize elastin via autofluorescence. The hADMSC/SMC co-culture experimental group produced a similar elastin network as the TGF- β 1 treated positive controls. The conditioned media showed a less developed elastin network than the hADMSC/SMC co-culture group and positive control group. While the negative control did show elastin production in the ninhydrin assay, a developed elastin network was non-existent in the autofluorescent images. The results of this study show promise for using hADMSCs as a possible elastogenic therapy, stimulating new elastin production by adult SMCs in vivo - ideally in the context of elastolytic diseases such as aneurysms. Interestingly, the underdeveloped elastin network seen in the conditioned media group may indicate that the co-culture of hADMSCs and SMCs allow the cells to communicate and better form an elastic network compared to growth factor treatment alone.

17. Non-invasive identification of treatment-responsive HER2+ breast cancer subtypes through DCE-MRI textural analysis

Nathaniel Braman, Prateek Prasanna, Salendra Singh, Donna Plecha et al

Case Western Reserve University

Background HER2+ breast cancer is biologically and clinically heterogeneous. PAM50 profiling of HER2+ breast cancer reliably identifies the HER2-Enriched (HER2-E) subtype as most responsive to HER2-targeted antibody therapy (trastuzumab). As this subtype is currently only identifiable using molecular profiling of breast tumor tissue, a non-invasive HER2-E identification method could reduce future patient morbidity. We present initial findings involving a new computational imaging feature (CIF) that captures the disorder among pixel level gradient directions on dynamic contrast-enhanced (DCE) MRI. We show that this CIF is able to discriminate between HER2-E and other HER2+ breast cancer subtypes on DCE-MRI. Methods 25 baseline DCE-MRI HER2+ breast cancer cases were split into two cohorts by PAM50-confirmed subtype (10 HER2-E, 15 non-HER2-E). 13 CIFs were computed on peak contrast image within expert-annotated lesions. This feature set was narrowed down to the 8 most differentially expressed features. Pharmacokinetic (PK) parameters (Kep, Ktrans, Ve), which quantify tumor-associated permeability changes, were also computed using Toft's model. Consensus clustering was performed using 1) CIFs and 2) PK parameters and peak intensities. Results CIFs yielded two distinct clusters, corresponding to HER2-E and non-HER2-E groups, with 70% sensitivity and 60% specificity. Studies sorted to the HER2-E cluster were characterized by under-expression of entropy and over-expression of texture homogeneity features relative to those of the non-HER2-E cluster. In contrast, PK parameters and peak intensities produced poorly defined groups that were uncorrelated with HER2 subtypes. Conclusion CIFs more accurately identified HER2-E status as compared with standard PK parameters on DCE-MRI. CIF expression patterns in HER2-E tumors suggest a less disordered imaging phenotype than other HER2+ tumors. Future validation studies of the HER2-E CIF signature identified here could potentially enable non-invasive prediction of response to trastuzumab.

18. Speed Versus Accuracy Tradeoffs in SensoriMotor Control and its Neural Correlates

Macauley S. Breault, Matthew S.D. Kerr, Pierre Sacré, Sridevi V. Sarma et al

Johns Hopkins University

Purpose: The speed-accuracy trade-off (SAT) is well known in phenomenon motor control in which one must exert more focus to complete a task accurately and subsequently at a slower rate. For example, attempting to accurately trace a line in one quick pass will most likely result in a flawed reproduction. Though a well studied topic, the neural basis of how the brain controls these tradeoffs is not yet understood due to lack of data. Several brain regions, including motor cortical areas, are involved in motor control and yet it is not possible to record from all regions simultaneously in humans as they make movements. To bypass this data limitation, several models of motor control have been developed and generally posit that the cerebellum acts as a feedback controller while motor cortical areas act as a feedforward controller. We hypothesize that fast movements implement feedforward control whereas slow movements incorporates feedback and as such involve different brain regions. **Methods:** Our study involved medically refractory epileptic patients implanted with several depth electrodes for clinical purposes. Brain activity was recorded using stereoelectroencephalography (SEEG) electrophysiological data at a sampling rate of 2 KHz from deep and peripheral brain structures. Data was collected on 8 subjects performing speed and goal-directed center out motor task using a robotic manipulandum and a computer screen. This system allowed for precise tracking of arm movements over a horizontal plane as the subjects manipulated a cursor presented on the screen along with the task stimuli. The behavioral task was to move and maintain their cursor from the center (represented by a circle in the center of the screen) to a target (a circle either left, right, above, or below the center) within an instructed fast or slow time (scaled from calibration results in which subjects were told to move to right target from center as quickly as possible). Each trial comprised of a instructional/priming phase (visual time instruction (fast or slow), move to center, target presentation), the movement phase (Go cue, reach and hold cursor on target), and a feedback phase (actual movement duration, reward (visual \$5) or failure (big red X) comparing actual time to instructed time). Perturbations were performed but were not considered for this analysis. Behavioral analysis was performed on trajectories between leaving the center and first hitting the target during the movement phase of un-perturbed trials. **Results:** Preliminary analyses of the behavioral data shows that trajectories associated with fast movements yield consistent paths, while those associated with slow movements produce correlated paths. This observation suggests that the subject may be adjusting their trajectory to reach the target optimally, possibly due to feedback over the longer trial period. Trajectories also varied based on directionality and subjects varied in their approaches to meeting the time constraint. **Conclusion:** Differences were observed between fast and slow movements in the behavioral data analysis with differences also seen across directions. The SEEG data may reveal the underlying neural differences between these fast and slow movements.

19. Chromosome refolding model of mating-type switching in yeast

Gabriel Bronk, Dr. Barış Avşaroğlu, Kevin Li, Dr. James E. Haber et al

Brandeis University

Chromosomes are folded into cells in a non-random fashion, with specific genes occupying well-defined spatial regions. This observation raises the question whether chromosome functions such as transcription and recombination are determined by its spatial organization. We consider this general question in the specific context of mating-type switching in budding yeast, which is a model system for homologous recombination. Mating-type switching is induced by a DNA double-strand break (DSB) at the MAT locus on chromosome III, followed by homologous recombination between the cut MAT locus and one of two donor loci (HMLa and HMRa), located on the same chromosome. Previous studies have suggested that chromosome III in MATa cells undergoes refolding after the DSB that directs recombination to the HMLa donor. Here we propose a quantitative model of mating switching predicated on the assumption of DSB-induced chromosome refolding, which also takes into account the previously measured stochastic dynamics and polymer nature of yeast chromosomes. Using quantitative fluorescence microscopy, we measure changes in the distance between the donor (HMLa) and MAT loci after the DSB, and find agreement with theory. Predictions of the theory are also in agreement with measurements of changes in the usage of HMLa as the donor, when we perturb the refolding of chromosome III. These results establish refolding of yeast chromosome III as a key driving force in MAT switching and provide an example of a cell regulating the spatial organization of its chromosome so as to direct homology search during recombination.

20. An in vitro System to Model the Effects of Fibrosis on Liver Development

Matthew Brovold, Shay Soker

Wake Forest Institute for Regenerative Medicine

Purpose: Congenital liver disorders are rare, but devastating diseases that can culminate in the need for pediatric liver transplant. The cause for these diseases remains unknown, and animal models for these diseases remain imperfect and expensive. Many congenital liver disorders result in a profibrotic environment generated largely by activated hepatic stellate cells (HSC), which produce large amounts of cytokines such as TGF- β and extracellular matrix (ECM) proteins such as collagen. TGF- β and non-activated HSC have both been shown to be critical during liver development. Perturbations in TGF- β signalling can result in biliary duct malformations, hallmark of many congenital liver disorders. As HSC produce large amounts of TGF- β when activated it follows that the increased production of TGF- β by the activated HSCs could cause biliary duct malformations. Our lab has developed a unique in vitro model capable of recapitulating processes of biliary tubulogenesis. Here we propose to use this system to model the effects of the fibrotic environment during development. Methods: Liver organoid models were produced by seeding decellularized liver matrix (DLM) disks with either primary fetal liver progenitor cells or immortalized liver progenitor cells (HepaRG). Primary HSC or LX-2 cells were in several ways: (1) HSC's or LX2 only (control), (2) HSC's or LX2 + HepaRG on plastic and on DLM, (3) HSC's or LX2 + HepaRG in a transwell for 1-2 weeks. HepaRG cells were analyzed for changes in gene expression of pathways critical during development using qPCR. Changes in the physical structure of any tubule formation were analyzed with Confocal IFC. Results: Cocultures resulted in a variation of gene expression in progenitor cells as compared to controls. HSC's also displayed variation of gene expression when cultured on different substrates plastic vs DLM. Structural changes in tubule formation were not able to be seen most likely due to time restrictions of the co-culture model. Conclusions: HSC's are affected by substrate, illustrating the need for materials that mimic the in vivo micro-environment. Activated HSC's do influence gene expression of liver progenitor cells in this model system. A larger analysis of additional developmental such as OPN, TBX3, Notch etc. should be conducted in the future and compared to tissue samples from patients who suffer from these liver disorders. Further comparisons will need to be conducted to determine if the changes in expression were due to TGF- β produced by aHSC's, environmental TGF- β or autocrine signalling.

21. [18F]3F4AP: a new PET tracer for demyelinating diseases

Pedro Brugarolas

The University of Chicago

Purpose: Central nervous system demyelination represents the pathological hallmark of multiple sclerosis (MS) and is thought to contribute to a variety of other neurological conditions including traumatic brain injury, stroke and Alzheimer's disease. The ability to quickly and quantitatively assess demyelination is crucial for the diagnosis and treatment of these diseases. As current imaging approaches for demyelination rely on magnetic resonance imaging, which is neither quantitative nor specific for demyelination, we have explored the possibility of targeting axonal potassium (K⁺) channels to image changes in myelination by positron emission tomography (PET). These channels, which normally reside beneath the myelin sheath, become exposed upon demyelination, resulting in leakage of intracellular K⁺ ions and disruption of action potentials. Consequently, the K⁺ channel blocker 4-aminopyridine (4-AP) is used clinically to enhance axonal conduction in MS patients. Here, we demonstrate that an F-18 labeled derivative of 4-AP can serve as a PET tracer for imaging changes in myelination. **Methods:** - Ex vivo C-14 autoradiography to evaluate the tracer distribution in the brain of mouse models of demyelination - Biochemical assays to find fluorinated 4AP analogs and characterize their binding affinity and stability - Radiochemical synthesis methods to prepare [18F]3F4AP - PET imaging and gamma counting in rodents to characterize the tracer in vivo **Results:** Using C-14 autoradiography, we found that 4AP and the fluorinated derivative, 3F4AP, show significantly higher uptake in demyelinated over normally myelinated white matter and can be used to distinguish demyelinated versus control mice. A method for preparing [18F]3F4AP was developed comprising of aromatic nucleophilic substitution followed by reduction. microPET/CT imaging in healthy rats showed that the tracer localizes primarily in non-myelinated areas of the brain with a mean whole brain SUV (0-30 min) of 3.93. Additionally, microPET/CT and autoradiography studies in demyelinated rats showed that [18F]3F4AP can be used to detect demyelination in vivo non-invasively. **Conclusion:** We have developed the first PET tracer whose binding increases in demyelinated brain regions. [18F]3F4AP is metabolically stable and is based on an approved MS drug. Thus, this compound is a promising PET tracer for demyelinating conditions.

22. Development of an In Vivo Bone Fatigue Damage Model using Axial Compression of the Rabbit Forelimb

Evan G. Buettmann, Matthew J. Silva

Washington University in St. Louis

Purpose: Many nontraumatic fractures seen clinically in patients with metabolic bone disorders or on antiresorptive treatment show an increased incidence of microdamage accumulation and impaired intracortical remodeling. Currently, the non-invasive forelimb fatigue model in rodents represents the “state of the art” system to study the biological and mechanical factors governing microdamage accrual and repair in bone. However, the lack of basal remodeling and Haversian bone in rodents limits their translatability in studying bone damage repair mechanisms. The work presented here demonstrates the development of the forelimb loading model in rabbits, the smallest mammal with intracortical Haversian remodeling. **Methods:** The forelimbs of post-mortem adult female New Zealand white rabbits were loaded in axial compression to determine their basic monotonic (0.5mm/sec displacement ramp) and fatigue (2 Hz haversine) properties. In addition, single element strain gauges were applied circumferentially around the average longitudinal location of ulnar fracture in order to determine the local bone deformation profile. Following time zero characterization, stress fractures were created in vivo and animals were allowed to recover for a period of two or five weeks. Formation of damage and new bone following mechanical loading was assessed by x-ray, microCT, and dynamic histomorphometry. **Results:** The rabbit forelimb when loaded in axial compression demonstrates a consistent mid-diaphyseal fracture location characterized by a local mixed compression-bending loading environment. Forelimb apparent stiffness, when fatigue loaded, demonstrates a progressive increase until macrocrack formation (+30%), at which time apparent stiffness rapidly declines until complete forelimb failure. Stress fractures in the rabbit ulna display robust periosteal expansion and woven bone formation two weeks following fracture. Five weeks following fracture, remodeling is seen around the fracture line and within the woven bone. **Conclusion:** Time zero characterization of the mechanical environment of the rabbit forelimb under cyclic axial compression allowed us to successfully create stress fractures in vivo. Survival animals experienced no complications associated with loading, demonstrating the efficacy of our model. Stress fracture creation triggered periosteal bone formation and an intracortical remodeling response, as seen in rodents. These biological findings support further development of this fatigue model as a means to study the effects of pharmaceuticals on the creation and removal of damage in osteonal bone.

23. Light-Triggered Release of Bioactive Molecules from DNA Nanostructures

Susie S. Cha, Richie E. Kohman, Xue Han

Boston University

Rapid advances in DNA nanotechnology allow the creation of intricate nanostructures that can be functionalized with a high degree of spatial control. To advance the use of DNA nanotechnology for controlled release of bioactive molecules, we report a general strategy using light to liberate encapsulated cargoes from DNA nanostructures with high spatiotemporal precision. We designed a multilayered, brick-like nanocage structure with a well-defined cavity in its center containing 14 addressable, single-stranded DNA extensions. Nanostructures were self-assembled in a single step by slowly cooling a heated mixture of DNA components. Cargo molecules were prepared by reacting with photolabile cross-linker and subsequently conjugating to oligonucleotides that are complementary to those presented in the cavity of nanocages. TEM revealed properly assembled structures with the desired shape and a clearly visible central cavity. Photocleavage of the cross-linkers was validated by conjugating an oligonucleotide to the fluorescent molecule Oregon Green (OG). 50% of OG was released after 11s and complete cleavage was achieved after 40s of low-powered light irradiation. To quantify loading efficiency, the activated OG was loaded into the nanostructures which incorporated a nonlabile dye for comparison. UV absorbance spectra analysis showed two distinct absorption peaks corresponding to the two dyes with 7.4 to 1 ratio, suggesting half of the DNA extensions on each cage were bound. We then explored the possibility of releasing large proteins from the nanocages, using bovine serum albumin (BSA) and streptavidin. TEM analysis of nanostructures revealed clearly visible BSA and streptavidin proteins within the cavity of the nanocages. Loading efficiency of 93% for BSA and 71% for streptavidin was observed. After low power light irradiation for 60s, we found uncaging efficiencies of 79% for BSA and 87% for streptavidin. To demonstrate that molecules released from the DNA nanocages retain their bioactivity, we tested uncaging of the small molecule glutamate by measuring calcium changes in cultured neurons mediated by the neurotransmitter using fluorescence imaging. Before light illumination, little basal calcium activity was observed. Immediately following a 1 ms light pulse illumination, we observed an increase in intracellular calcium levels in 16.22%. In the absence of the DNA nanocages, no cells exhibited a change in calcium levels upon light. In conclusion, we describe a novel strategy to encapsulate a large variety of bioactive molecules inside DNA nanostructures that can be released with high temporal precision using pulses of light.

24. Multi-focused HIFU for diffusive heating and control of acoustic cavitation

Vandiver Chaplin, Charles Caskey

Vanderbilt University

1. Background Thermal therapy with high intensity focused ultrasound (HIFU) requires controlled elevation of temperature in tissue and typically uses array transducers that enable electronic steering and beamforming. However, peak negative pressures (PNP) used in thermal therapy are capable of causing spontaneous cavitation, which can accelerate heating but also poses a risk of mechanical damage due to inertial cavitation. A method that reduces PNP while delivering a therapeutic thermal dose is desirable. Here, we present a multi-focused technique that can produce a volumetric heating rate equal to or greater than a single-focused technique, while reducing cavitation by operating at a lower PNP. 2. Methods We implemented multi-focus sonication consisting of a triangular focal pattern with 3 foci 4-mm apart and compared it to the native single focus on the Philips Sonalleve V2 MR+HIFU system. To assess cavitation and heating abilities of each sonication scheme, we sonicated an agarose phantom with known absorption and thermal characteristics (2% agarose, 1.5% graphite). We first measured cavitation activity at non-thermal energy levels (1.2 MHz, PNP range of 0.3-8.5 MPa, repetition frequency of 1Hz and 1ms duration) by analyzing echoes received on a 10MHz single-element transducer. The cavitation signal was isolated by filtering harmonics and integrating broadband noise components. We then compared heating during continuous multi- and single focus sonications at matched power using MR thermometry by measuring the peak and average temperature change in an 8x8x9-mm ROI. 3. Results After cavitation onset, broadband spectral energy associated with cavitation was greater in single- versus multi-focus sonications at all power levels tested, confirming that multi-focus sonication reduces cavitation activity. During thermal experiments at matched power, the temperature rise in the multi-focus case reached a maximum of 47°C compared to 88°C for the single focus case; importantly, both strategies yielded an average heating rate of 24 deg C / min. Our findings demonstrate that multi-focal sonication can be used to reduce cavitation without sacrificing volumetric heating rate in thermal therapy, minimizing undesirable effects of cavitation.

25. A Novel Bioluminescent Split Reporter Strategy for Investigating the Regulation of SNAP29 Homodimerization in Starvation-Induced Autophagy

Ian Y. Chen, Eric Marceau, Thillai S. Veerapazham, Junfeng Ma et al
Stanford University

Background—Autophagy plays an important role in cell survival under starvation. Recent evidence suggests that the synaptosomal-associated protein 29 kDa (SNAP29) regulates starvation-induced autophagy by coordinating autophagosome-lysosome fusion. Current mechanistic model of SNAP29 function fails to capture the fact that SNAP29 exists as both homodimers and monomers. Here, we report the development of a novel bioluminescent split reporter-based sensor for investigating the regulation of SNAP29 monomer-dimer equilibrium during starvation-induced autophagy. **Methods and Results**—A bioluminescent sensor for detecting SNAP29 homodimerization using a split firefly luciferase (FLuc) fragment complementation strategy was specifically constructed. This sensor is composed of a single plasmid vector construct (pUbi-N-FLuc-SNAP29-pUbi-SNAP29-C-FLuc) expressing two fusion proteins—SNAP29 fused to the N-terminus of FLuc (N-FLuc) and SNAP29 fused to the C-terminus of FLuc (C-FLuc)—each under the regulation of a constitutive human ubiquitin promoter (Ubi). Human cervical cancer (HeLa) cells expressing the bioluminescent sensor and exposed to starvation in Earle's Balanced Salt Solution (EBSS) medium showed decreasing homodimerization/FLuc signal over a 3 hour period ($27\pm 3\%$ at 3 hour, $p<0.05$), during which there was decreasing amount of SNAP29 sugar modification via O-GlcNAcylation by Western blot. Global knockdown of O-GlcNAcylation for 24 hours in these cells by siRNA targeting O-linked N-acetylglucosamine transferase (OGT) resulted in significantly less homodimerization/ FLuc signal at baseline and during starvation ($24.8\pm 4.3\%$ over 3 hours, $p<0.05$). HeLa cells expressing a mutant version of the sensor in which the wild-type SNAP29 was replaced by a mutant O-GlcNAcylation-resistant SNAP29 (SNAP29QM) likewise showed less homodimerization/FLuc signal at baseline and during starvation ($36.5\pm 3.5\%$ over 3 hours, $p<0.05$). **Conclusions**—O-GlcNAcylation regulates SNAP29 function and autophagy by shifting the monomer-dimer equilibrium towards homodimers. Correspondingly, the reduction of SNAP29 O-GlcNAcylation during starvation favors SNAP29 monomerization that is needed for effective autophagy. With further refinement, the bioluminescent SNAP29 homodimerization sensor can provide a sensitive and convenient platform by which small molecules/therapeutics can be screened for their ability to modulate autophagy by affecting the SNAP29 monomer-dimer equilibrium.

26. Investigating feedback circuits with 2D intestinal organoid models

Weilin Chen, Curtis Thorne, Steven Altschuler, Lani Wu

University of California, San Francisco

The intestinal epithelium is a highly dynamic tissue that carries out important digestive functions. Multiple cell types compose the intestinal epithelium and are derived from the intestinal stem cells located at the bases of the crypts. Despite a fast turnover rate, the epithelium does a remarkable job of maintaining its cell populations. To study this phenomenon, we are developing an ex vivo model of intestinal crypts. We combine this system with high-resolution image profiling at the single-cell level to build a model of the signals that regulate proliferation and establish tissue homeostasis.

27. Portable robot for autonomous intravenous access using 3D near infrared and ultrasound imaging

Alvin I. Chen, Max L. Balter, Timothy J. Maguire, Martin L. Yarmush
Rutgers University

Purpose: Venipuncture is the most commonly performed invasive clinical procedure and leading cause of medical injury in the United States. Complications are exacerbated in difficult settings, where the rate of success depends heavily on the practitioner's skill and the patient's physiological condition. Described here is the development of a portable robotic device that improves the accuracy and speed of venipuncture by drawing blood and delivering intravenous fluids in an autonomous manner. **Methods:** The device is designed for use in large hospitals, diagnostic labs, and primary care settings, where complications due to difficult venous access greatly compromise the safety and quality of care. The device combines near infrared and ultrasound imaging, real-time computer vision and pattern recognition software, and a miniaturized nine degrees-of-freedom robotic manipulator. The underlying technology operates by mapping the 3D position of a selected vein and precisely guiding the needle into its center under closed-loop image and force feedback. **Results:** In multiple feasibility studies, the device has demonstrated significantly improved vein detection in humans compared to trained manual visualization, and 98% accuracy in 35 sec on commercial and customized tissue phantoms simulating human physiological characteristics over a broad demographic range. Animal and human clinical trials have been initiated, and work is underway to couple the device with on-board microfluidics-based assays to allow critical diagnostic information to be obtained rapidly and at the point of care. **Conclusion:** Compared to current clinical standards, automated venipuncture may provide healthcare professionals the ability to draw blood and start IV lines with unparalleled accuracy and speed, and furthermore may remove the practitioner from contact with exposed sharps, thus eliminating the transfusion risk. Once translated, the described device has the potential for adoption in a number of arenas including pediatric, geriatric, emergency, and military use. The technology furthermore represents a step in the miniaturization and automation of robotic systems for routine medical interventions.

28. Long-Term Stability of Stimulating Multi-Contact Nerve Cuff Electrodes on Human Peripheral Nerves

Breanne P. Christie, Max Freeberg, Kevin M. Foglyano, Michael E. Miller et al
Case Western Reserve University

Introduction: Nerve cuff electrodes (NCEs) have been applied to femoral nerves to evoke hip and knee movements in motor system neuroprostheses for standing and stepping after paralysis [Fisher 2008]. The purpose of our study was to determine the chronic stability of multi-contact NCEs in human neuroprosthesis recipients in terms of charge threshold, joint moment, and selectivity for multiple years after implantation. Methods: Stimulation was delivered via four-contact spiral NCEs, implanted bilaterally on the femoral nerves of two volunteers with SCI 5-6 years ago. Repeated stimulated responses were measured with a 6-DOF load cell on a dynamometer, with the knee fixed at 20° of flexion while pulse width-modulated twitch recruitment curves were generated for every contact. Pulse widths were between 0-255µs and amplitude was 0.8-2.1mA. Mean maximum and just noticeable twitch moments (10% maximum) were derived from Gompertz models fit to the data. Charge threshold was defined as the minimum charge required at each contact to produce a just noticeable twitch moment. Linear regression was utilized to quantify change in moment and charge threshold over time. Two contacts that elicited adverse sensation in Subject 2 were removed from analyses. Selectivity is being quantified by repeatedly determining the optimal stimulation parameters that produce the largest moments while minimizing overlap in muscle recruitment [Fisher 2013]. Results: The mean maximum twitch moment across all contacts was 0.10±0.01Nm/kg for Subject 1, and 0.12±0.02Nm/kg for Subject 2. Between both subjects, the moment produced by 11/14 contacts exhibited no change over time, while the remaining three decreased ($p < 0.05$) to 36.68±3.74% of the original moment. Activating multiple contacts simultaneously produced tetanic moments 4x the 0.135Nm/kg needed to stand [Kagaya 1998]. Average charge threshold across all contacts was 78.16±44.8nC for Subject 1 and 42.15±2.66nC for Subject 2. Seven contacts showed no change in charge threshold over time, 5/14 exhibited a significant decrease, and 2/14 increased ($p < 0.05$). One contact in Subject 1 increased by 83.3nC between the first and last session, and one contact in Subject 2 increased by 31.2nC. Even the highest threshold was well within safe levels multiple years after implantation. Conclusion: The stimulated responses of NCEs in terms of twitch recruitment and charge threshold appear to be consistent and stable for up to six years after implantation in two human subjects. As muscles strengthen and contractile properties change, stimulus parameters may need periodic re-optimization at intervals that are being determined in ongoing analyses of stability of selectivity.

29. Characterization of contraction intensity differences in strain development during isometric muscle contraction

Crystal L. Coolbaugh, John E. Mendoza, Bruce M. Damon

Vanderbilt University

Noninvasive quantification of regional muscle deformation (e.g. strain and strain rate) may aid the interpretation of structural and functional alterations in muscle-tendon mechanics associated with performance, injury, and disease. Attempts to apply cardiac magnetic resonance tagging techniques to skeletal muscle, however, have required constrained experimental preparations – numerous contractions at low contraction intensity – that limit the functional relevance of the acquired data. Harmonic phase (HARP) analysis may improve extraction of muscle motion from tagged images, but it is unclear if this technique can be applied to skeletal muscle during higher intensity contractions. Purpose: To use HARP analysis to measure the magnitude and spatial pattern of strain in the tibialis anterior (TA) muscle during submaximal and maximal isometric contractions. Methods: Spatial modulation of magnetization (SPAMM) tagged images were acquired in the axial and sagittal planes in the TA muscle of eight healthy volunteers during isometric ankle dorsiflexion contractions at 25, 50, 75, and 100 % of maximal voluntary contraction intensity. Two contractions were performed at each intensity level to assess strain measurement repeatability. HARP analysis was used to measure the dynamic three-dimensional displacement and strain field in a region bisecting the internal aponeurosis of the TA for each trial. Results: Our preliminary analysis indicated the direction of strain was consistent with the bulk muscle movement observed during an isometric contraction of the TA. Strain magnitudes across the aponeurosis also related positively with muscle contraction intensity except for the 100 % intensity condition, which may suggest a possible strain rate limitation of HARP analysis. Conclusions: This study demonstrates the utility of HARP for analyzing in vivo skeletal muscle mechanics under conditions experienced during activities of daily living. Future work will extend these imaging techniques to other clinically relevant muscle groups (e.g. gastrocnemius and hamstring) in previously injured or older subject populations.

30. Fluorescently-tethered Hsp90 inhibitors provide therapeutic effect and diagnostic information in breast cancer pre-clinical models

Brian Crouch, Stella Belonwu, Helen Murphy, Philip Hughes et al

Duke University

Purpose: The purpose of this study is to establish that a single agent can be used to both visualize and treat breast cancer. Heat Shock Protein 90 (Hsp90) is a molecular chaperone that stabilizes and protects many 'client' proteins necessary for tumor growth. We have previously developed a fluorescently-labeled Hsp90 inhibitor (Hs-27) that binds to ectopically expressed Hsp90 on the surface of cancer cells. In this study, we demonstrate the theranostic utility of Hs-27 by treating breast cancer cell lines and examining therapeutic endpoints, as well as examining uptake of Hs-27 in these same cell lines in vitro and a subset in vivo. **Methods:** Hs-27 combines an Hsp90 inhibitor (SNX-5422) with a fluorescein isothiocyanate derivative (excitation 488nm / emission 525nm). To establish the therapeutic capabilities of Hs-27, three breast cancer cell lines were treated with Hs-27 and analyzed for client protein expression through western blotting. As many Hsp90 client proteins regulate cellular metabolism, metabolic endpoints were also investigated using a Seahorse Extracellular Flux Analyzer. Hs-27 uptake was evaluated both in vitro and in vivo. Three breast cancer cell lines were incubated with Hs-27, and fluorescence images were captured using confocal microscopy. An image processing method was used to evaluate these images and establish differences in Hs-27 uptake between various breast cancer receptor subtypes. In vivo uptake of Hs-27 was investigated using hyperspectral fluorescence imaging of a dorsal skinfold window chamber model for mice with either breast tumors or no tumor as a control. **Results:** Western blotting of breast cancer cells treated with Hs-27 revealed Hsp90 client protein degradation consistent with other potent Hsp90 inhibitors. Additionally, Hsp70 expression increased as a compensatory method as Hsp90 was inhibited. Consistent with decreased Hsp90 client protein expression, both glycolysis and oxidative phosphorylation were significantly lower in breast cancer cells. In vitro confocal microscopy revealed that Her2 overexpressing cells take up more Hs-27 than either estrogen receptor positive or triple negative cell lines. In vivo imaging of window chambers demonstrated that Hs-27 uptake is greater in tumor bearing mice than non-tumor bearing mice. **Conclusions:** In this study we provide compelling evidence that Hs-27 has both therapeutic and diagnostic utility in a variety of breast cancer subtypes. Hs-27 causes degradation of client proteins and causes metabolic changes in breast cancers that may precede cell death. Finally, Hs-27 uptake is greater in tumor mice than non-tumor mice, highlighting its utility as a diagnostic tool.

31. Functional Electrical Stimulation for Restoration of Proprioception

Ivana Cuberovic, Emily L. Graczyk, Matthew A. Schiefer, Dustin J. Tyler

Case Western Reserve University

Proprioception is important for executing movements. Individuals, such as amputees, without proprioception rely on vision for movement feedback, which increases cognitive burden during task performance. Any disruption to vision significantly degrades performance with the prosthesis. Thus, prostheses are often relegated to supporting functions. This study reports on work to provide functional, intuitive proprioceptive feedback with direct neural interfaces. Two experiments were conducted with one unilateral trans-radial amputee who had Flat Interface Nerve Electrodes (FINEs) implanted on his median and radial nerves in January 2013. Electrical stimulation of the median FINE resulted in sensation of hand motion. The subject mirrored his phantom hand's position with his intact arm. A CyberGlove™ system captured changes in joint angle. Electromyography (EMG) recording of the residual muscles in the amputated limb indicated concomitant level of muscle activation. The subject verbally reported the sensation quality. Stimuli were biphasic with constant amplitude (0.7 mA) and frequency (100 Hz). Stimulation phase duration varied from one to five seconds. In one session, four pulse width (PW) envelopes were used: flat; ramp up then down; ramp up then hold; and ramp up then off. In the second session, only the flat PW envelope was used, but its amplitude was set to one of five evenly distributed levels between sensory threshold (120 μ s) and the hardware maximum (255 μ s). In both, EMG envelopes were found by bandpass filtering, rectifying, integrating, and normalizing to a maximum voluntary contraction. Calibrated joint angle traces were extracted from CyberGlove™ data. The presence of proprioception and EMG were strongly associated (Fisher exact test $p < 0.001$). Further, the shape of the pulse width envelope, the evoked EMG, and measured joint motion are strongly positively correlated. The mean normalized cross-correlation coefficient between stimulation PW envelope and EMG was 0.90 ± 0.15 , PW envelope and joint angle was 0.84 ± 0.13 , and EMG and joint angle was 0.77 ± 0.20 . The EMG signal was quantified by normalizing the area under the curve (AUC) of the EMG envelope by trial duration. The perceived motion was quantified by the measured range of motion. Both duration and PW affect the magnitude of the evoked EMG and extent of perceived motion, where PW variation led to a larger dynamic range of perceived motion and better correlation (linear regression $r^2 > 0.95$).

32. Non-monotonic temporal evolution of gradient-echo MRI signal in brain white matter

Kyle S. Decker

Duke University

Purpose: Gradient-echo signal is traditionally assumed to decay exponentially. However, this signal may not always decay exponentially if the underlying field distribution is not Lorentzian. We show through simulation and ex-vivo MR results that the field distribution for gradient-echo images may not follow a Lorentzian curve in the presence of ordered structures such as fiber bundles located within white matter (WM) of the central nervous system. **Methods:** Simulations of a WM fiber bundle were performed in order to investigate microstructural effects on the observed signal decay. A susceptibility tensor was assigned to each grid point in the simulation and the forward Fourier transform relationship between susceptibility and magnetic field was used to calculate the magnetic field distribution of the voxel for a range of echo times. T2 decay was incorporated into the simulation through the use of a 3-pool model. Ev-vivo images of C47BL/6 mouse brains were acquired on a 9.4T 89-mm vertical bore Oxford magnet. Two groups of mice with different cuprizone diets were scanned (n=2, total of 4 mice). One group of mice was administered a cuprizone diet for 4 weeks and then allowed to recover for 8 additional weeks. This group serves as a remyelination group. The second group was administered the cuprizone diet for the entire 12 weeks, thus serving as a chronic demyelination group. **Classification of non-monotonic decaying voxels** was performed using regularized logistic regression, which was trained on a simulated dictionary of monotonic and non-monotonic decaying signals. **Results:** In the simulation at 9.4T, the field distribution becomes significantly broad at larger fiber angles, causing deviation from a Lorentzian curve and resulting in non-monotonic signal decay. Similar results are seen as the field strength is increased and the g-ratio is decreased, both resulting in non-Lorentzian field distributions and non-monotonic signal decay. The non-monotonic decay was seen in voxels throughout the major WM regions of the ex-vivo mouse brain. The incidence of the non-monotonic decay in WM ROIs was significantly higher in the remyelination group in comparison to the chronic demyelination group. **Conclusion:** At high field strengths the observed gradient-echo signal does not decay as expected in areas with sufficient myelination and relatively large fiber angles with respect to B0. We found the magnitude actually increases for certain echo times. The observed phenomena are highly indicative of the underlying microstructure and thus may be a useful tool for studying cytoarchitecture both in vivo and ex vivo.

33. Improved quantification of drug delivery using MRI quantitative susceptibility mapping

Kofi Deh, Marjan Zaman, Pascal Spincemaille, Moonsoo M. Jin, Yi Wang,
Weill Cornell Medical College

Quantifying the accumulation of drugs or contrast agents (CA) in a subcutaneous tumor implant in an animal model is a common application of magnetic resonance imaging (MRI) in cancer drug research. Several MRI data acquisition and post-processing techniques have been developed to facilitate this procedure. Generally, however, these techniques do not account for contributions of non-CA sources, such as the background field, fat, uncertainty in r_2 relaxivity, and T1 effect, to the measured CA concentration. The increase in the use of molecular drug targets, however, creates the need for more accurate quantification of drug delivery. Here we investigate the accuracy of quantitative susceptibility mapping (QSM), which accounts for the non-CA contributions to CA concentration measurement. 5 SCID mice bearing PC3-PIP tumors in the flank were injected either intra-tumorally or intravenously with a dual-modality iron oxide/zirconium contrast agent and scanned on both Positron Emission Tomography (PET) and 7 Tesla MRI scanners. QSM images are reconstructed from MRI phase images and compared to PET images. We observed excellent agreement between QSM and PET estimates of drug accumulation in the tumors. We contrast estimates from QSM with estimates obtained using R_2^* relaxometry to highlight problems with using the latter approach.

34. Adaptation of the Dichromic Fluorescence (DCF) strategy to study kinase activation

Elizabeth N DeLassus, Duanwen Shen, Mingfeng Bai, Baogang Xu et al

Washington University St. Louis

The signaling status of different kinases can be an important indicator of cellular homeostasis since kinases regulate many important pathways in the cell. Aberrant kinase signaling is implicated in many pathologies including heart disease, diabetes and cancer. Therefore, the ability to monitor kinase activity in real time would be a powerful tool in imaging disease states in which these enzymes are implicated and monitoring response to drugs that modulate kinase activity. Research in the Achilefu lab has demonstrated that inducing structural asymmetry in cypate using different adducts including peptides leads to a shift in emission from 800nm to 700nm thus generating dichromic fluorescent molecules. Synthesis of an asymmetrical cypate derivative with the structure phosphoserine-cypate-serine (LS455) yields a dichromic molecule which has enhanced 700 nm emission compared to cypate. When the serines conjugated to cypate are symmetrical the resulting molecule LS456 has maximum emission similar to cypate at approximately 800nm. However, in vitro phosphorylation of LS456 by the kinase Akt-1 in MCF-7 breast cancer cells generates asymmetry in the molecule and results in enhanced 700nm emission with a concomitant decrease in 800nm emission. This shows that the DCF strategy is a viable way to image kinase activity with potential to be expanded to image this diverse family of important enzymes.

35. Characterizing and Eliminating Errors in Enhancement and Subtraction Artifacts in DCE MRI Studies

Jamal J. Derakhshan, Elizabeth S. McDonald, Evan S. Siegelman, Mitchell D. Schnall et al

University of Pennsylvania

Purpose: Dark band artifacts were observed in dynamic contrast enhanced (DCE) MRI subtraction images routinely used clinically to diagnose breast cancer and remained unexplained, causing a diagnostic dilemma, despite efforts by both site MR physicists and vendor technicians at optimizing the acquisition, including using different fat suppression techniques. It was hypothesized that the artifacts were caused by a subtle change in signal cancellation between fat and water in the presence of contrast enhancement. A related phenomenon termed paradoxical suppression had been previously reported on opposed-phase non-subtraction images only. Methods: Computer simulations were performed in Matlab for voxels containing 0-100% fat fraction at all off-resonance angles, assuming 50% enhancement for glandular tissue and 10% for fat. Phantom experiments were performed by using mixtures of methylene chloride (similar chemical shift to water) and fat with chromium acetylacetonate (hydrophobic) as a doping agent to approximate the T1 of glandular tissue and simulate enhancement. A time-equivalent volume interpolated breath-hold examination (VIBE) acquisition was engineered by increasing Partial Fourier in both phase and partition-encode directions. Phantom experiments were performed using a standard ACR quality control phantom to explore the high contrast spatial resolution and low contrast detectability of the modified sequence. Finally, a standard minimum TE/TR clinical VIBE DCE MRI study was bracketed by the modified time-equivalent in-phase VIBE sequence in a clinical DCE MRI study. Results: Simulations demonstrated and characterized the large variable reduction in expected enhancement as a function of off-resonance angle and fat signal fraction. Phantom experiments validated the simulations showing reduction of enhancement up to -160%. Fat-suppressed VIBE reduced fat signal to near water level. ACR phantom experiments demonstrated improved high contrast spatial resolution and low contrast detectability in the in-phase time-equivalent sequence. The in-vivo studies demonstrated elimination of the subtraction artifacts when imaging with a time-equivalent, in-phase acquisition. Conclusion: Errors in enhancement and subtraction artifacts in contrast-enhanced MRI studies are an important clinical problem and have been completely characterized for the first time (chemical shift artifact of third kind). These are present despite optimal fat suppression and both can be eliminated by imaging with a time-equivalent in-phase acquisition.

36. A Microfabricated Submucosa for Assessing the Effects of Matrix Topography on Colorectal Cancer

Mahesh Devarasetty, Aleksander Skardal, Shay Soker

Wake Forest Institute for Regenerative Medicine

Recent investigation of colorectal cancer metastasis has identified the tumor microenvironment as a large proponent of metastasis. Factors such as tissue stiffness, fiber alignment and bundling, and cell-cell interactions have been targeted as affecting cancer cell phenotype, proliferation and drug susceptibility. To study these effects in vitro, this study aims to develop a micro-facsimile of colonic submucosal microstructure using cellularized type I collagen (Col I) hydrogels. Constructs are fabricated using rabbit colonic smooth muscle cells (RCSMCs) suspended in a Col I hydrogel. Although RCSMCs were able to produce contraction throughout a range of Col I concentrations, RCSMCs demonstrated increased propensity for aligning fibrillar ECM components in lower concentration Col I hydrogels. To assess the effects of RCSMC remodeling on cancer cells, we embedded a foci composed of HCT-116 cells (a malignant colorectal cancer cell-line) into the submucosal construct. HCT-116 cells produced mesenchymal phenotypes in stiffer, high concentration Col I hydrogels, and also demonstrated more epithelial expression when cultured in submucosal constructs indicating a connection between smooth muscle cells, fiber alignment, and cancer cell phenotype. To decouple paracrine activity from fiber effects induced by the RCSMCs, we performed transwell co-cultures and inhibited fiber alignment using beta-aminopropionitrile (a lysyl oxidase inhibitor). In both cases, cancer cells demonstrated amplified mesenchymal expression. Our results indicate that RCSMCs remodel the extracellular matrix into a “normal” or healthy environment that induces “normal” or epithelial expression from HCT-116 cells. Future directions include probing the biomolecular effects of fiber alignment as well as the integration of cancer-associated fibroblasts into the model to replicate the cellular content found in in vivo tumors.

37. Implanted Myoelectric Prosthetic Control for Transradial Amputees

Hendrik A Dewald, Matthew R Williams, Joris Lambrecht, Robert F Kirsch

Department of Biomedical Engineering, Case Western Reserve University

Purpose: Our work focuses on the implementation of implanted myoelectric signals (MES) acquisition systems for control of multi-degree of freedom (DOF) prosthetic hands for individuals with transradial amputation. Major limitations of existing myoelectric control in prosthetics stems, in part, from surface MES recording electrodes. Existing myoelectric prostheses use surface electrodes to employ a “two-site, two-state” control algorithm capable of driving one DOF at a time. More advanced multi-DOF hands require more, and often simultaneous, commands to provide a more natural and intuitive interface. Numerous intramuscular electrodes will provide a more stable and selective acquisition system for the machine learning approach of an Artificial Neural Network (ANN). The overall goal of this study is to create a robust and simultaneous multi-DOF prosthetic control scheme using machine learning techniques and a consistent set of implanted electrodes for specific and independent MES signals.

Methods: Temporary intramuscular fine wire electrodes are used in both control and amputee subjects to record MES activity during a set of cued finger and wrist isometric flexion/extension tasks. Electrodes are inserted in pronator teres, supinator, extensor pollicis longus (or abductor pollicis longus), flexor pollicis longus, flexor digitorum superficialis, extensor digitorum communis, flexor digitorum profundus, and extensor digiti minimi. The collected MES data is used to train an ANN, which is then used to control a 3 DOF virtual hand in a series of posture-matching tasks. The ANN control performance is compared against a simpler 6-site agonist-antagonist control that serves to mimic the “two-site, two-state” methodology of existing myoelectric prostheses. Time to target, simultaneity, and path efficiency are measured and compared across the two approaches. Results: Subjects exhibited 100% posture-matching task completion with little to no significant performance difference between agonist-antagonist and ANN control approaches. Additional experiments are ongoing to evaluate a larger and more complete data set.

Conclusion: In our work, we demonstrated successful virtual hand postural control, both using ANN and agonist-antagonist methods. Future work beyond the acute fine wire phase will entail the use of permanently implanted intramuscular recording electrodes in the amputee subject group.

38. Alterations in the anterior capsule correlate with impaired joint mechanics in a rat elbow model of post-traumatic joint contracture

Chelsey Dunham, Ryan M. Castile, Necat Havlioglu, Leesa M. Galatz et al

Washington University in St. Louis

PURPOSE Post-traumatic joint contracture (PTJC) due to elbow injury is a challenging clinical problem due to the anatomical and biomechanical complexity of the elbow. Injury to the elbow disturbs joint congruity and the peri-articular soft-tissue leading to decreased range of motion (ROM). Recently, our group developed an animal model of PTJC in the rat elbow. We evaluated our model biomechanically and morphologically to determine if it mimicked symptoms common to human PTJC. **METHODS** In this IACUC approved study, male Long-Evans rats had unilateral elbow surgery to replicate soft-tissue injuries seen in human dislocation followed by immobilization for 42 days. Limbs were evaluated at 3, 7, 21 and 42-day immobilization (IM) and 42-day remobilization (RM). Control animals were allowed unrestricted cage activity. Elbow joints were subjected to mechanical testing in flexion-extension and pronation-supination. After mechanical testing, sagittal sections of elbow joints from 42-day IM and RM were stained with hematoxylin and eosin (H&E), toluidine blue and picrosirius red for histological assessment. **RESULTS** In flexion-extension, injured limbs at 42-day IM and RM demonstrated significantly decreased ROM compared to control and contralateral limbs. In pronation-supination, injured limbs at 42-day IM and RM showed significantly decreased ROM compared to contralateral. Preliminary data for injured limbs in flexion-extension was only significantly different from contralateral for 21-day IM; 3 and 7-day IM was not significantly different. H&E staining of injured limbs at 42-day IM revealed an increase in the amount of adhesions, cellularity and thickness of the anterior capsule compared to control and contralateral. While at 42-day RM, the capsule from injured limbs exhibited increased adhesions and thickness; however, cellularity decreased. Toluidine blue had a similar trend for cellularity. Picrosirius red staining showed an increase in anterior capsule collagen density for injured limbs at 42-day IM and RM. **CONCLUSION** We have shown that our animal model of PTJC mimics biomechanical and biological features of the human condition. Significant motion loss persisted from 42-day IM to 42-day RM, likely due to the increased disorganized collagen and thickness of the anterior capsule. Preliminary data showed that significant motion loss developed between 7 and 21-day IM. Biological evaluation at these earlier time points is currently under investigation. We are also studying how other peri-articular soft-tissues, besides the capsule, contribute to motion loss. Understanding the mechanobiological signaling of peri-articular soft-tissue will help identify specific changes in the elbow responsible for contracture.

39. No changes necessary: Zaire Ebolavirus efficiently infects and replicates in Boa Constrictor cells without cytopathic effect.

Greg Fedewa, Melissa Spear, Ryan Hernandez, Sheli Radoshitzky et al

UCSF

Purpose: Ebola virus disease is a type of hemorrhagic fever with a high mortality rate, no cure, no approved vaccination and no approved treatments. Caused by members of genus Ebolavirus, little is known of these viruses in the wild, including their natural reservoirs. The glycoprotein (GP2) of reptile Arenaviruses, which infect both boa constrictor and pythons, possesses both sequence and structural similarity to Ebolavirus GP, suggested that Ebolavirus may also be able to infect and replicate in boa constrictor cells. **Methods:** To test this, we serially passaged Zaire ebolavirus strain Kikwit "R4368" in a boa constrictor cell line, JK, in parallel to passage in HeLa cells. We then deep sequenced the six serial passages to characterize genomic mutations associated with adaptation for growth in JK cells and identify genomic locations under positive selection. We also performed anti-GP antibody staining of the passages to characterize viral growth. **Results:** We observed that Zaire ebolavirus will infect JK cells, yet caused no obvious cytopathic effect. Further, we found that serial passage of Zaire ebolavirus in JK cells resulted in no mutations being required for infection and replication. Deep sequencing coverage (>10,000x) demonstrated the existence of 48 low frequency variants within the initial inoculum viral population and a mean of 70 variants found per passage. **Conclusion:** When we attempted to passage Marburg Marburgvirus, despite having a similar range of possible hosts, it was not able to infect the JK cell line, indicating JK is not just a permissive cell line. The lack of cytopathic effects is also a hallmark for a reservoir species. Egyptian Rousette Bats (*Rousettus aegyptiacus*) were long thought to be the reservoir for Zaire Ebola virus until it was recently shown that they don't become viremic when experimentally infected. Taken together with our data, we believe that additional species should be considered as possible reservoirs this pathogen.

40. Biomedical and Cosmetic Applications of Electrochemical Polyion Sensors

Stephen A. Ferguson, Mark E. Meyerhoff

University of Michigan

Heparin is a common anticoagulant used in clinical procedures to prevent clotting during surgical procedures (e.g., open heart surgery). Conversely, protamine is used to neutralize the anticoagulant effect of heparin in the extracorporeal circuit after a surgery is complete. The concentrations of these highly charged macromolecules (polyions) are difficult to detect and quantify directly in blood. To circumvent this challenge, electrochemical polymer membrane-based polyion sensitive ion-selective electrodes (ISEs) have been developed as tools for the detection/quantification of heparin and protamine in simple background electrolytes as well as in whole blood. Further, polymeric quaternary ammonium salts (polyquaterniums) represent a class of compounds that have found increasing use in industrial and cosmetic applications. In particular, polyquaterniums are useful in industrial flocculation processes, such as wastewater/drinking water clarification, as they form aggregates with oppositely charged species in colloidal solutions, thus allowing the combined weight of the aggregate to settle out of solution. Also, polyquaterniums have been found to be useful as conditioners and antistatic agents in personal care products such as shampoos. In this presentation we will summarize research efforts aimed at developing simple electrochemical/potentiometric polyion detection methods to quantitate concentrations of various polyions in solution and their potential application to microfluidic devices. One approach is based on titrating samples with oppositely charged polyions and detecting excess titrant polyion via a titrant polyion-sensitive membrane electrode. Another approach is to mix a given volume of sample to a flowing stream of oppositely charged polyion, and measure the decrease in response of a reversible potentiometric polyion sensor toward the indicator polyion owing to it being bound by the analyte polyion. These new methods may provide a means to determine levels of heparin/protamine concentrations in whole blood during clinical procedures in addition to providing a quality control method for quantitating polyquaterniums in personal care product formulations.

41. Metabolomics approaches towards a spatial understanding of host-microbe interactions in plants

Dimitrios J. Floros, Pieter C. Dorrestein

UCSD

Purpose Ubiquitous and diverse microbial communities together with their associated genomes make up microbiomes that are often linked to plant and human health outcomes. However, the investigation of the metabolites associated with these communities and their effects on host metabolome have not been adequately characterized. Better understanding of these communities are needed for their rational engineering and utilization in the clinic or for sustainable agriculture. My research aims to incorporate analytical chemistry together with emerging bioinformatic and visualization tools to begin ascribing functionality and causal relationships to the members of microbe-host communities. **Methods** While sequencing based Omics approaches, like 16S amplicon and transcriptomic libraries, allow us to understand the composition and some of the function of microbial communities, it is changes in the metabolomes that often play the largest part in defining different phenotypes and pathologies. Untargeted high resolution tandem mass spectrometry (MS/MS) based metabolomics allows the rapid generation of testable hypotheses about metabolites that may play key roles in these communities. Through our lab's rapidly maturing molecular networking platform large MS/MS datasets can be readily analyzed and interpreted, while new visualization tools for LC-MS/MS data are making it possible to generate 3D maps of the metabolome. **Results** We have developed methodologies of 3D spatio-chemical visualization of microbe-host interactions, which has allowed the observation of microbial specialized metabolites, like valinomycin, on plant surfaces. Additionally, molecular networking tools have allowed the comparison of plant species and tissues at the metabolite level, including level 2 and 3 annotations (Metabolomics Standards Initiative). When applied to plant metabolites of members of the Solanum family, these techniques allowed the rapid comparison and visualization of tissue-type dependent molecular distributions. **Conclusion** This work has successfully developed techniques for layering molecular information onto three dimensional models. Molecular networking was also successfully applied to mine the MS/MS datasets for plant and microbial metabolites. As these studies continue into controlled multi-member communities we will apply these MS-based metabolome mapping tools to understand the direct effectors and impact of microbial communities on plant metabolism.

42. Quantitative Microscopy of Dopamine Receptor Signaling in Pancreatic Beta Cells

Daniel J.P. Foust, Brittany Caldwell, Antoine G. Godin, Alessandro Ustione et al

Washington University

Purpose We are interested in resolving the molecular signaling mechanisms and the subsequent physiological responses that mediate insulin secretion in pancreatic beta cells. Impaired insulin secretion is indicated in the progression of diabetes mellitus. Bettering our understanding of the molecular interactions that modulate insulin secretion could lead to new therapeutic interventions. Specifically, our lab has previously shown that activation of D2-like dopamine receptors acts to inhibit insulin secretion. These receptors are coupled to trimeric G-proteins on the plasma membrane. When the receptor is activated, it catalyzes the dissociation of the trimeric G-protein into separate G-alpha and G-beta-gamma subunits. The dissociated G-alpha and G-beta-gamma subunits interact with downstream effectors to initiate the physiological response. The molecular details of interactions between dopamine receptors, G-proteins, and their effectors are not known in beta cells. Studying these interactions could be source of novel therapeutic targets for diabetes. **Methods** We have labeled D2-like receptors and the G-beta-gamma subunit with fluorescent proteins. These recombinant proteins are heterologously expressed in MIN6 cells, a murine beta cell line. We image these cells using single and two-photon excitation. Imaging parameters are selected so that data may be analyzed with various statistical analysis tools. These tools belong to family methods referred to as fluorescence fluctuation spectroscopy (fluorescence correlation spectroscopy, photon counting histogram, image correlation spectroscopy, etc). In addition to previously developed analyses of fluorescence fluctuations we have developed spatial intensity distribution analysis for detection of fluorescence in two channels (2D-SpIDA). This novel method gives additional sensitivity to heteromeric protein-protein interactions. **Results** Initial results have shown a decreased affinity between D2-like dopamine receptors and the G-beta-gamma subunit upon dopaminergic stimulation. **Conclusion** Fluorescence fluctuation spectroscopy is a useful approach for measuring the interactions of dopamine receptors with their downstream effectors in beta cells.

43. Measuring growth patterns during neonatal brain development with surface strain analysis

Kara E. Garcia, Emma C. Robinson, Dimitrios Alexopoulos, Cynthia E. Rogers et al

Washington University in Saint Louis

Purpose: During the final trimester of fetal development, the human cortex undergoes dramatic deformation to produce complex folding patterns. Abnormal folding is associated with disorders including autism, schizophrenia, and epilepsy, yet the folding process remains poorly understood from a physical standpoint. To evaluate the proposed mechanisms of folding, precise measurements of spatiotemporal growth are needed. Using strain as our metric, this study seeks to measure spatiotemporal patterns of cortical surface deformation during critical stages of neonatal brain development. **Methods:** In collaboration with the Saint Louis Children's Hospital, cortical surface reconstructions were generated from magnetic resonance imaging (MRI) at postmenstrual age 26-38 weeks. Multimodal Surface Matching (MSM) was used to automatically align surfaces based on mean surface curvature (Robinson et al. 2014). To minimize nonphysical strains, we implemented a new MSM functionality based on the theory developed by Knutsen et al. 2010, which minimizes strain energy by considering the brain surface as a compressible neo-Hookean material. After alignment, strain maps were calculated from vertex motion between the younger and older cortical surfaces of an individual subject. For detailed visualization of strain magnitude and direction, surfaces were exported into MATLAB for post-processing. **Results:** Using MSM to align surfaces and minimize anatomical surface strains, we were able to (1) precisely match gyri and sulci from an individual across multiple time points and (2) estimate growth patterns for an individual during brain development. Inclusion of strain relaxation reduced maximum strains more than three-fold, such that all strains fall within the physically realistic range. In general, we observed higher strains in regions where gyri are forming, or in sharp sulci. However, it is worth noting that some of these variations, particularly in the sulci, may reflect artifact due to sharp curvatures that increase tendency toward data alignment. **Conclusions:** The strain information presented here is the first of its kind for human cortical development in the preterm brain. As such, this analysis may provide valuable physical measurements to evaluate proposed hypotheses and models of cortical folding. While no "ground truth" data exist to validate our results, future work will test MSM output against cases for which the true deformation is known. In future studies we may also align surfaces based on alternative data (sulcal depth, fMRI) or compare growth patterns across multiple subjects to ascertain trends in normal and abnormal development.

44. Ion Channels Bound to Endogenous Ferritin are Sensitive to Radiofrequency Waves

Eric Gibbs,

Duke University

Several research groups, including our own, have recently reported that when the iron-storage protein ferritin is bound to the Ca²⁺ ion channels TRPV1 or TRPV4, the resulting channel is sensitive to static magnetic fields and/or radiofrequency waves (RF). RF or magnetic-sensitive ion channels fill an immediate void in neuroscience and developmental research, where present methods of ion channel control require invasively perturbing the in-vivo system. RF weakly interacts with biological tissue but is readily absorbed by magnetic materials. Ferritin is a protein that accumulates and stores iron as a super-paramagnetic nanoparticle. The effects of exposing ferritin to low levels of RF are negligible to the cell as a whole, but our group hypothesized that by localizing ferritin to an ion channel, RF/ferritin interactions could influence ion channel behavior. We localized ferritin to TRPV1 and TRPV4 ion channels to test this hypothesis. TRPV1 and TRPV4 are both Ca²⁺ ion channels that are important in sensing a cell's physical environment. Ferritin was localized to TRPV1/TRPV4 using a short peptide sequence (120 amino acids) that promotes ferritin recruitment to ion channels (FeRIC). This sequence is taken from the high-weight molecular kininogen, which binds to ferritin during inflammation. Our group has done several in-vitro and in-vivo studies that demonstrate FeRIC TRPV1 and TRPV4 respond to RF and minimally perturb cell physiology. The gold-standard in ion channel studies is electrophysiology but the RF used to open FeRIC channels interferes with electrophysiology signals so GCaMP imaging was used to study increased Ca²⁺ activity in the presence of RF. After confirming that FeRIC channels were activated in the presence of RF, we used the channel to investigate congenital heart defects in chicken embryos. It was previously reported that congenital heart defects in chick embryos were caused by heating the egg at a critical point in development. It has also been shown that such defects are caused by irregular migration of neural crest cells (NCCs). Our group hypothesized that temperature-induced activity of TRPV4, which expresses in NCCs during development, causes irregular migration and heart defects. Using FeRIC TRPV1, we showed that defects could be induced at a high rate (11/23) when electroporated into NCCs and exposed to RF. WT channels and RF led to no defects while only 1 of 22 embryos not exposed to RF but expressing FeRIC TRPV1 had a defect. This has important implications for maternal fever and congenital heart defects in humans.

45. Translational Research in Biomaterials (TRB)

Mark W. Grinstaff,
Boston University

The mission of the Translational Research in Biomaterials (TRB) training grant is to develop PhD students into interdisciplinary and translational research scientists / engineers. Through their TRB training, they will acquire: 1) a fundamental and quantitative understanding of materials, polymer chemistry, surface science, biomaterial-tissue response, and molecular and cellular biology; 2) exposure to engineering technologies and characterization techniques; 3) research experience in interdisciplinary programs that promote discussion and scientific inquiry in areas outside of the student's "comfort zone"; and 4) training in societal impacts of new technology, ethics, clinical trials, and basic business. The cornerstones of the TRB program are the curriculum and the program elements that combine interdisciplinary research, quantitative science and engineering courses, translational-based courses in clinical trials and business, student-organized seminar club, dinners with a medical doctor, training in professional ethics, individual development career plans, and professional / career development workshops. These skills are essential in future biomedical careers as graduates join teams with diverse backgrounds that strive to meet a common goal in research, development, and, ultimately, commercialization.

46. A POMDP Framework for Breast Cancer Screening Decisions

Simon X. Han, William Hsu, Alex Bui

UCLA

Purpose. Breast cancer is the second most common cause of death from cancer in women in the United States. The most reliable and effective way to reduce death is early detection and treatment. Routine mammograms are the current recommended method of detection for most women. The recommendations, however, differ in terms of screening frequency, starting age, and ending age, depending on the institution and its implementation of the process. Recent research also further suggest an overdiagnosis and overtreatment trend in breast cancer, increasing the burden on patient and healthcare system alike. These all point to the need to better understand screening frequency. In this work, we leverage a large breast cancer screening dataset at our institution and apply a decision making framework to formalize optimal screening schedules. **Methods.** Information was extracted from our institutional electronic health record (EHR) system. Patient data includes mammogram results, biopsy results, and previous diagnoses. We then used this data to instantiate a partially observable Markov decision process (POMDP), a stochastic decision making framework, to provide insight into “optimal” policies for screening frequency. In a POMDP, an agent makes sequences of decisions (policies) that produce the best probabilistic outcome over time on behalf of the patient. The probabilities are learned from the patient data at our institution, considering trade-offs between early cancer detection and disadvantages of screening (e.g., false positives). The agent keeps track of patient history by maintaining belief over the status of the patient, with the belief updated as additional observations about the patient are made over time. Policies are adjusted as beliefs are updated as well. **Results.** We solve the POMDP as a finite-horizon discrete-time problem and derive a finite state controller (policy graph), which models the decision making process. From the policy graph, we generate a decision tree which can quickly inform a user (e.g., a radiologist) to select the optimal action based on an observation. Preliminary results suggest a biennial (once every other year) screening schedule is appropriate for the lowest risk patient population. **Conclusion.** We utilize a POMDP framework to explore screening mammogram schedules in breast cancer and demonstrate that it can suggest actions that optimize patient outcome. We anticipate that by keeping track of patient history and patient-specific observations over time, we can ultimately provide tailored recommendations for the individual patient.

47. Multi scale immune profiling of human peripheral blood with single cell RNA-Seq for immune system monitoring

Graham Heimberg, John Haliburton, Hanna Retallack, Eric Wong et al

UC San Francisco

Human peripheral blood contains one of the most important and dynamic components of host defense, the immune system. Within the immune system different cell types interact to maintain homeostasis and respond to environmental stimuli like drugs or antigens. Immune responses trigger transcriptional changes across multiple scales, modulating the expression of individual genes, entire transcriptional programs, or even rapidly expanding specific populations of cells. Here, we report a novel approach to profiling the immune system by using single cell mRNA-seq to capture information about immune activity across these three distinct scales. We show that the high information content of single cell mRNA-seq allows functional immune signatures to be extracted from sampling just thousands of cells from peripheral blood. We use data from healthy human donors and ex vivo disease models to computationally design a compact set of gene expression features that systematically describe immune responses. With this method for unbiased multi scale immune profiling we are able to positively identify systemic lupus erythematosus from peripheral blood, identifying known and novel biomarkers in a disease with no definitive diagnostic. This general approach to immune monitoring could lead to more quantitative and general diagnostic tools and may engender a new modality for precision medicine.

48. Beyond the EPR Effect: Multi-targeting Strategies of Nanoparticles to Image Invasive Glioma

Elizabeth Doolittle, Peter Bielecki, Efstathios Karathanasis

Case Western Reserve University

Purpose Typically, targeted nanoparticles treat tumors as monoliths. While cancer cells evolve, they alter their gene expression patterns and behavior over time and space, including the expression of targetable cell-surface biomarkers. Brain tumors display a dynamic microenvironment with spatiotemporal heterogeneity. Thus, a targeting nanoparticle system must take under consideration that a brain tumor is a collection of microenvironments. Dispersive glioma cells select “permissive” structures and pathways (perivascular growth along blood vessels or migratory paths across white matter tracts). Considering that glioma-associated vasculature is not as leaky as in other tumor types, we employ multi-ligand vascular targeting schemes against vascular biomarkers that effectively direct nanoparticles to primary and invasive glioma sites. Methods Imaging studies were performed using orthotopic murine models of glioma. A patient derived pediatric high-grade glioma (SJ-GBM2) and a patient derived adult glioma stem cell glioma (T4121). Brain tumors were generated using tumor cells expressing green fluorescent protein reporter gene (GFP). As a case study, liposomes, an all-purpose nanoparticle, were conjugated with different targeting ligands and NIR fluorophore for particle detection. Injection of cocktails containing nanoparticle variants into the same mouse allowed for comparison of targeting while controlling for variability in tumor growth and dispersion. Particles with separate fluorochromes, one targeted and one non-targeted, were intravenously injected. Ex-vivo fluorescence imaging of the brains was completed at a short time point (hours after injection) to reflect targeted accumulation on a small time scale, and at a long time point (1-2 days after injection) allowing for maximum passive (non-targeted) deposition. Organs were analyzed using 2D and 3D fluorescence imaging. Histological analyses confirmed whole organ imaging data and provided topological details. Results Surface ligands were added at varying surface densities (1000-25000 ligands per particle), confirmed by direct protein assay. Imaging studies showed that at a short time scale, targeting achieved enhanced particle delivery to the brains when compared to non-targeted variants. Notably, different targets differentiated targeting of the primary site or infiltrating edges and distant invasive sites. Based on quantitative measurements of whole brain imaging and histological analyses, performance of different targeting schemes will be presented. While EPR-driven delivery can result in significant particle accumulation in the well-vascularized primary tumor location, deposition of nanoparticles is patchy and near-perivascular. However, multi-targeting schemes facilitated efficient targeting to dispersive and invasive sites. Conclusion The spatiotemporal differences of brain tumors require careful consideration in order to design a nanoparticle targeting strategy to successfully image dispersive glioma.

49. Characterizing embryonic stem cell derived multipotent lung stem cells

Amritha Kidiyoor, Sean V Murphy, Anthony J Atala

Wake Forest Institute for Regenerative Medicine

Introduction: The lung parenchyma consists of many different types of mature lung epithelial cells, some of which are involved in cell replacement during normal turnover and after injury. Regeneration of epithelium has been observed by adult lung stem cells such as basal cells, Clara cells, bronchioalveolar stem cells (BASCs) as well as alveolar type II cells. These cells have limited differentiation potential. For example, basal cells can differentiate into lung epithelial cell types found in the upper airways whereas BASCs can differentiate into lung epithelial cell types found in the terminal airways and alveoli. The current theory supports the presence of highly proliferative multipotent lung stem cells in the lung bud tips in the pseudoglandular stage of lung development that can give rise to all the different airway and alveolar lung epithelial cell types. The objective of this study is to identify the potential of embryonic stem cells to give rise to a phenotype representative of the multipotent lung stem cell population found in the developing lung. **Methods:** Differentiating ESCs into multipotent lung stem cells- Embryonic stem cells (ESCs) were differentiated towards lung epithelium in a step-wise manner mimicking embryonic lung development. RNA was collected from cells at all stages of differentiation. **Characterizing the multipotent lung stem cell phenotype-** RNA collected from cells during the intermediate stages of differentiation was used for qPCR analysis of pulmonary and stem cell markers and a RNA microarray. Lung epithelial markers were also detected by immunofluorescence (IF) on cells at this stage. **Results:** ESC derived cells expressed markers characteristic of each differentiation stage indicating successful differentiation. Further, the ESC derived lung progenitor stage cells were found to express lung epithelial markers such as CFTR, CCSP, FOXJ1, MUC5AC by PCR, microarray and IF analyses. **Conclusion:** We were able to successfully differentiate ESCs into lung epithelium in a step-wise manner through an intermediate immature lung cell stage. Cells at this intermediate stage expressed multiple lung epithelial as well as stem cells markers at the mRNA and protein level indicating a multipotent lung stem cell phenotype.

50. Tracking Neural Activity In-Vivo using Polarization

Nathaniel O. King,

Washington University in St. Louis

Birefringence has been used to track changes in neurons via transmission illumination for decades. The changes in the birefringent properties of these neurons has been shown to track with neural activity. Additionally, the requirement that the light source and imager be on opposite sides of the neural tissue means that this work has only been conducted in-vitro. Using a novel, division of focal plane, sensor we have developed the first in-vivo prep, allowing us to track changes in the polarized component of reflected light. Tracking activity in the antenna lobe of locusts we found changes in polarization state that align with olfactory stimulus. In addition to the olfactory derived responses, we observe a wave that propagates at 0.5Hz. We are continuing to investigate the limits of resolution for these odor evoked responses as well as the origins of these slow moving oscillations.

51. Microvascular Engineering: Recapitulating the Bone Marrow Niche

Surya Kotha, Amie Adams, Brian Hayes, Kiet T Phong et al

University of Washington

Purpose: Hematopoietic cells dynamically interact with their surrounding microenvironment during their residence, maturation, and differentiation. Individual marrow components have been isolated in 2D in vitro cultures, yet their functional contributions to a complete niche are not fully understood. In vivo studies are complex, and the inaccessibility of marrow architecture has precluded systematic analysis of each component. Here, we employ an in vitro 3D microfluidic system to study hematopoietic cell trafficking in an engineered vascular niche. **Methods:** Our system allows for control of 3D geometric cues, hydrodynamic flow, multi-cellular compositions, and cellular matrix remodeling by combining soft lithography and injection molding in type I collagen gel (Zheng et al. PNAS 2012). Endothelial cells perfused through the embedded microfluidic network form a confluent, patent endothelium within the collagen. Incorporating hematopoietic cells and fibroblasts within the collagen allows visualization of cell interactions with vasculature. To create a competent marrow niche in vitro, we embedded cells from fresh human bone marrow screens within the collagen matrix. Next, we developed a simplified marrow fibroblast niche by incorporating two different human marrow-derived fibroblast cell lines (HS27a and HS5) in the collagen to understand how the marrow microvasculature is influenced by endothelial phenotype. **Results:** In our human marrow platform, we found that the heterogeneous cell fraction could be cultured in for at least two weeks. Electron microscopy showed various cells interacting with and changing the endothelium. Throughout perfusion culture, we identified different cell populations were released into the circulation over the course of two weeks, including CD34+ hematopoietic progenitor cells, megakaryocyte, erythroid, lymphoid, and myeloid lineage cells. In our simplified marrow fibroblast platform, we observed that HS27a and HS5 cells created distinct microenvironments by secreting divergent inflammatory cytokine profiles. Both stromal lines reduced endothelial expression of vWF and junctional proteins. These modified vessels yielded distinct adhesion and extravasation patterns to perfused monocytes and CD34+ stem cells. **Conclusions:** In summary, we developed an in vitro 3D microvascular marrow niche and gained insight into hematopoietic cell trafficking between the matrix and the circulation. By guiding the interplay of heterogeneous cell populations, we have demonstrated the capacity to define distinct microenvironment spaces. This platform shows promise for long term culture of a whole marrow population and for the ex vivo generation of hematopoietic cells. Further development of this 3D marrow platform will further our understanding of the complexities mediating stem cell trafficking, residence, and differentiation in health and disease.

52. Cell-free compartmentalized protein synthesis inside double emulsion templated liposomes with in vitro synthesized and assembled ribosomes

Jin Woo Lee, Filippo Caschera, Kenneth K.Y. Ho, Michael C. Jewett et al

University of Michigan

Purpose: Assembling biological parts into a functional system using a bottom-up in vitro reconstitution approach offers the possibility of designing artificial cells with the ability of sensing and responding to external stimuli. Artificial cells are defined as the encapsulation of biologically active material in a biological or synthetic membrane. We describe a robust and general method to produce artificial cells for the purpose of mimicking one or more behaviors of a cell. **Methods:** A cell-free expression platform for making bacterial ribosomes encapsulated within giant liposomes was capable of synthesizing sfGFP. The liposomes were prepared using a double emulsion template, and compartmentalized in vitro protein synthesis was analyzed using spinning disk confocal microscopy. Two different liposome phospholipid formulations were investigated to characterize their effects on the compartmentalized reaction kinetics. **Results:** At first, we encapsulated the cell-free expression system in DOPC based liposomes in buffer but we did not observe any noticeable synthesis of sfGFP. We hypothesized that this was due to the fact that some of the important small molecules needed for protein synthesis could cross the membrane. We therefore carried out the cell-free expression reactions encapsulated by double emulsion template in a feeding solution that includes energy components, which led to protein synthesis. When DOPC was switched to POPC, a less permeable lipid, sfGFP was generated when buffer was present. Also, a delay was observed in sfGFP generation when the feeding solution was present. **Conclusion:** We report the development of experimental conditions necessary to encapsulate the cell-free expression system into liposomes. This study was performed as a necessary step towards the synthesis of minimal cells. Looking forward, our approach may be integrated with more complex designs such as the expression of cytoskeletal and membrane proteins, and encapsulation of gene circuits.

53. Theranostic Viral Nanoparticles: From Imaging to Therapy

Karin L. Lee, Nicole F. Steinmetz

Case Western Reserve University

Trainee: Karin L. Lee Purpose: Each year, one million new cases of cancer are diagnosed, with the most aggressive being treated with chemotherapy. However, chemotherapy has side effects when administered systemically and is not effective for long-term treatment due to the development of resistant cells. Attachment of chemotherapeutics to nanoparticles decreases side effects, while also improving overall payload delivery and efficacy. Plant viral nanoparticles (VNPs) are being investigated as carrier systems to deliver therapies to specific cells and tissues. VNPs have symmetrical structures, are amenable to chemical modification, and can be produced in high yields in plants. Additionally, they are biocompatible, biodegradable, and non-infectious in mammals. Potato virus X (PVX) is a filamentous virus, measuring 515 x 13 nm and comprised of 1270 identical coat proteins, each containing a solvent-exposed lysine residue. As a filamentous nanoparticle, PVX offers a novel nanomaterial, since synthetic nanoparticles cannot be synthesized at high aspect ratios. Therefore, we must first investigate its interactions with biological systems. Here, using multiple imaging techniques, we evaluate the impact of surface modifications on the in vitro and in vivo properties of PVX, while also developing it for delivery of chemotherapy. Methods: PVX was modified with fluorescent tags, as well as polyethylene glycol or targeting molecules. For chemotherapy delivery, doxorubicin was attached. Modified particles were studied in vitro and in vivo using a combination of confocal and fluorescence microscopy, flow cytometry, and fluorescence molecular tomography (FMT). Results: We formulated PEGylated, targeted, or drug-loaded PVX particles. Using, FMT, we determined that PEGylated particles exhibited distinct biodistribution compared to native PVX particles; the difference in biodistribution correlated to different circulation profiles. In vitro, PVX particles targeted towards the epidermal growth factor receptor (EGFR) had specific uptake in EGFR+ cells in single and co-culture, as measured by flow cytometry and confocal microscopy. Lastly, doxorubicin was attached to PVX (PVX-DOX) and evaluated for cell efficacy. PVX-DOX had decreased efficacy compared to free DOX in a panel of cell lines, but maintained its cell killing ability. Using fluorescence microscopy, we determined that the decrease in efficacy was due to reduced uptake of doxorubicin after loading onto PVX. Conclusions: Here, we developed the filamentous plant virus PVX for use as a cancer therapy. We utilized multiple imaging techniques to evaluate its interactions with biological systems, with and without surface modifications. PVX offers a promising platform, with modifiable properties, for use in cancer therapy.

54. Molecular insights into vein remodeling with arterial flow: role of COUP-TFII

Li Li, Mercedes Balcells, Takaharu Ichimura, Ming Tao et al

Brigham and Women's Hospital

Purpose: Veins remodel under arterial flow. This occurs in a vein graft for coronary bypass surgery or arteriovenous fistulae (AVF) used in hemodialysis, and indeed such changes are required for graft utility. Maladaptation of the vein can lead to graft failure. Though well-defined the driving molecular mechanisms behind vein arterialization are not clear. We examined these events. Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII, also known as nuclear receptor subfamily 2, group F, member 2, NR2F2) is expressed in venous, but not arterial, endothelial cells. In addition serving as a specific venous endothelial cell marker, COUP-TF II plays an essential role in the fate decision of endothelial cells during development. This study investigates the expression and regulation of COUP TFII in AVF with the overall goal to achieve a better understanding of the molecular mechanisms that drive vein arterialization. **Methods:** Venous specimens were collected during revision of malfunctioning AVF from hemodialysis patients with end-stage renal disease. COUP TFII and activated Notch 1 staining were evaluated on these specimens using immunofluorescence. We also examined the effects of arterial flow on COUP TFII expression in venous endothelial cells in vitro. Cultured human saphenous vein endothelial cells were exposed to different shear stress (0, 5,10, 40 dyn/cm²) for 48 hours in controlled perfusion bioreactors. **Results:** Human venous segments from malfunctioning AVF showed significant intimal hyperplasia covered with intact endothelium. COUP TFII staining was positive on endothelial cells as expected. Furthermore, significant COUP TFII staining appeared in neointima, most expressed by endothelial cells in many neovessels in intimal hyperplasia. Activated Notch 1 expression was substantial in intimal hyperplasia, similar to the pattern seen with COUP TFII. In the cell culture system in the bioreactor, COUP TFII expression was observed in venous endothelial cells but was minimal in arterial endothelial cells. Under high shear stress (40 dyn/cm²), venous COUP TFII expression decreased significantly compared to levels seen in static venous endothelial cells. **Conclusions:** There is substantial endothelial cell expression of COUP TFII and activated Notch 1 associated with intimal hyperplasia in malfunctioning human AVF. In the bioreactor system in vitro, protein expression of COUP TFII was decreased in venous endothelial cells exposed to high shear stress. Dysregulation of COUP TFII and Notch pathways may be involved in AVF malfunction and vein graft failure. The different expression of COUP TFII with arterial flow between conditions in vivo and in vitro suggest that shear stress is not the only driving force for vein remodeling. The underlying mechanisms linking COUP TFII expression to vein arterialization requires further investigation.

55. Dissecting enhancer grammar in the developing *Drosophila*

Lily Li, Zeba Wunderlich

University of California, Irvine

During development, enhancers drive the expression patterns that specify cell fate decisions. The complexity of these decisions can be defined as the number of cell fates from which an enhancer has to choose. Thus, early in development, when cells are mostly homogeneous and are simultaneously differentiating into many different cell types, the enhancers are driving a relatively complex set of decisions; in contrast, later in development, when cells are more differentiated and are only making decisions between a few cell types, the enhancers are driving a much simpler set of decisions. Consequently, we expect that enhancer architecture, in terms of enhancer length, number of transcription factor binding sites (TFBSs), and average information content, will reflect the complexity of cell fate decisions being made. We first consider the differences in the architecture of enhancers in the *Drosophila* anterior-posterior (AP) and dorsal-ventral (DV) patterning systems. These systems have been extremely well-characterized, and they exemplify the disparity in decision complexity that enhancers need to drive, as the AP axis consists of 14 segments whereas the DV axis consists of six germ layers and sublayers. We then consider a larger data set of developmental enhancers and characterize changes in the architecture of the enhancers active over development. We find that regulation of more complex decisions is associated with increased enhancer length and number of TFBSs and with decreased average information content. This can be explained by the fact that increased numbers of TFBSs can be arranged in more ways, allowing for enhancers to drive more patterns of expression, and thus increased complexity. This examination of enhancer architecture in the context of cell fate decisions helps us understand why enhancer architecture is so diverse, with well-characterized enhancers containing as few as two to as many as fifteen TFBSs.

56. Label-Free High Throughput Microfluidic Device for the Isolation and Single Cell Multiplex Gene Expression Analysis of Circulating Tumor Cells from Breast Cancer Patients

Eric Lin, Lianette Rivera, Hyeun Joong Yoon, Shamileh Fouladdel et al
University of Michigan

The metastasis of cancer is preceded by the dissemination of cancer cells from the primary tumor site to remote sites via the blood circulation. The presence of circulating tumor cells (CTCs) in the peripheral blood represents a strong and independent prognostic factor for decreased disease-free and overall survival. Immune-affinity based capture, although being the most commonly used method for the isolation of CTCs, offers low throughput ($\sim 1\text{mL/hr}$) and have considerably cell loss caused by the heterogeneous expression of biomarkers on CTCs. Various label-free approaches utilizing the physical properties of CTCs have been developed to overcome the limitations. Here we present an inertial microfluidic-based separation technique for high throughput and label-free isolation of CTCs yielding the highest throughput with high CTC recovery and high blood cell removal among all the label-free technologies. The isolated CTC populations were further analyzed for single cell multiplex gene expression analysis. The separation of CTCs in the device is driven by two main forces: (i) inertial force that focuses the cells into streamlines, and (ii) drag force from Dean flow that migrates the focused cells to various positions based on size. Device is optimized with MCF-7 and Panc-1 cell line within PBS buffer solution and diluted blood, and is tested in patients with breast cancer on an average of 5 mL of whole blood processed through double devices in series. CTCs isolated were analyzed for tumor specific protein markers and genomic characterization is done using single cell analysis techniques. Samples are processed through the inertial microfluidic device and CTCs are enriched in second outlet based on size difference between CTCs and blood cells. Device is optimized to operate at an extremely high throughput of $2500\ \mu\text{L/min}$ with high recovery (greater than 90%) and high white blood cells (WBCs) removal (5 log orders). In patient samples, we identified CTCs in 38 of 40 (95%) breast patients with metastatic disease ($5.4\pm 4.6\ \text{CTC/mL}$) with low WBC contamination ($663\pm 647\ \text{WBC/mL}$). Based on the gene expression, both inter and intra patient heterogeneity of CTCs at the single cell level were discovered among the tested patient samples. The study of CTCs could have a direct impact upon society by presenting novel ways to address one of the major hurdles in cancer research – early detection – and will foster the advancement of science and engineering via the exploration of new druggable targets approaches and the further understanding of the pharmacodynamics.

57. Developing a bacterial surface display system for the generation of targeted outer membrane vesicles

Jessica S. Lin, Anton Bryksin, Wilbur Lam, Thomas H. Barker

Georgia Institute of Technology

Purpose: To develop and optimize a bacterial surface display system to display an antibody that binds selectively to fibrin over soluble fibrinogen, and to generate outer membrane vesicles that can be targeted to fibrin clots in wound sites. **Methods:** Reduced genome *E. coli* are transformed with vectors containing the PET autotransporter and the myc-tagged fluorescent protein mCherry. Flow cytometry with phycoerythrin labelled anti-myc antibody is used to characterize the presence of protein on the surface of the bacteria. Microscopy is used to confirm the localization of the protein to the periplasmic space. **Results:** Flow cytometry with a myc-tagged mCherry demonstrates that the myc tag is exposed on the surface of the cell only when the vector contains both the PET construct and a signal peptide directing the protein to the periplasmic space. Microscopy demonstrates that the mCherry is localized to the periplasmic space only in the presence of a signal peptide. **Conclusions:** The bacterial surface display system using the PET autotransporter displays protein on the cell surface and is a promising step towards the development of targeted outer membrane vesicles.

58. Quantifying the blood flow and oxygen metabolism responses to a neural stimulus: combining multiple MR methods to optimize the modeling of hyperoxia experiments

Eulanca Y. Liu, Jia Guo, David J. Dubowitz, Richard B. Buxton

UC San Diego

Purpose: To evaluate an approach for measuring cerebral metabolic rate of oxygen (CMRO₂) in both baseline and activation states from repeated activations in normoxia and hyperoxia, including effects of hyperoxia itself on CMRO₂, based on a simplified model for the BOLD signal. **Background:** Two general approaches to measuring baseline CMRO₂ are currently being investigated: measuring responses to hypercapnia and hyperoxia[1-3]; and isolating venous blood signal and measuring T₂, which depends on venous oxygenation[4]. Here we have applied both approaches, and used the measured data to test and refine a simplified model for the BOLD signal that includes changes due to CBF, CMRO₂ and hyperoxia. This extended data set makes it possible to estimate the model parameter alpha, and to estimate potential changes in CMRO₂ due to hyperoxia itself. **Experimental Methods and Results:** Eight subjects (4M/4F, mean age 25.8 yrs) were examined; CBF and BOLD responses measured with a dual echo spiral PICORE QUIPSS II ASL acquisition (TR=2500ms, T1=700ms, T2=1750ms, TE=3.3/30ms). The stimulus was an 8Hz flickering checkerboard, and an activated ROI in visual cortex was identified with a separate functional localizer experiment. Responses in 4 conditions were measured: 1) to hypercapnia (mean deltaPaCO₂=8.4mmHg); 2) to 60s activation; 3) to hyperoxia (mean deltaPaO₂=179mmHg); and 4) to activation during hyperoxia. A correction factor of 1.094 was applied to ASL measures of CBF in hyperoxia to correct for decreased T₁ of blood, based on previously reported values[5]. In separate experiments, baseline O₂ extraction fraction E₀ was measured with VSEAN[4] (mean value was E₀=0.41+/-0.097). For this study, BOLD and CBF responses were averaged across the subjects to obtain a high SNR dataset to use in evaluating the model and characterizing the physiological responses. **Modeling Results.** We tested a simplified model for the BOLD response based on the approximation that the BOLD signal is proportional to the absolute change in deoxy-hemoglobin (dHb), and that dissolved O₂ gas is always a small fraction of the arterial hemoglobin-bound O₂. This model approximates the more complete models developed in previous work[1-3]. In applying the model to the measured data we assumed: 1) No change in CMRO₂ with hypercapnia; 2) Activation deltaR (fractional change in CMRO₂) is the same in normoxia and hyperoxia; and 3) hyperoxia itself induces an additive change in CMRO₂. Assuming a value of alpha, the exponent for dHb weighted blood volume change, four model parameters were calculated from the four measured responses: M, a scaling factor (8.3%), deltaR-activation (16.6%), deltaR-hyperoxia (-3.8%), and wO₂ (a factor combining the change in arterial PO₂ in a hyperoxia experiment with baseline CBF and CMRO₂). From wO₂, E₀ was calculated with the assumption Hb=9.0 mM. We then used the independent measurement of E₀ using VSEAN as a constraint, making it possible to estimate alpha as well (0.11). **Conclusions.** A relatively simple BOLD signal model for quantifying CMRO₂ during baseline and activation was evaluated in the context of

measuring ASL/BOLD activation responses in both normoxia and hyperoxia. Using VSEAN, a separate measurement of baseline O₂ extraction fraction gave an average value of 0.41 in these subjects, in good agreement with literature values. This value was used in the modeling of combined ASL/BOLD data to allow a fit for the value of alpha (0.11). Because this model includes the effects of CMRO₂, CBF and deltaPaO₂, it is possible to fully characterize CMRO₂ without special equipment to force specific blood gas changes, even when there are associated changes in CBF and CMRO₂ induced by hyperoxia itself (estimated to be -9.2% and -3.8%, respectively, in this study). References. 1) Gautier and Hoge, Neuroimage 60:1212 (2012); 2) Wise et al, Neuroimage 83:135 (2013); 3) Blockley et al, Neuroimage 122:105 (2015); 4) Guo and Wong, MRM 68:1458 (2012); 5) Bulte, et al, NeuroImage 60:1 (2012). Acknowledgements. NIH grant support: NS036722 and NS085478

59. Polyethylene Glycol Hydrogel Microparticles for Drug Delivery

Allen L. Liu, Andrés J. García

Georgia Institute of Technology

Synthetic polyethylene glycol (PEG) hydrogel microparticles (microgels) encapsulating bovine serum albumin (BSA) are synthesized. A microfluidic synthesis platform is employed, affording good control over particle diameter and monodispersity. BSA is introduced into the aqueous PEG flow channel prior to droplet formation and microgel crosslinking. The BSA serves as an affinity-based macromolecule to better modulate release of a diffusion-loaded drug. Release of a fluorescent model drug loaded into our PEG microgels is investigated in vitro.

60. Implementation of a Clinical Ultrasound Coherence Imaging System

Will Long, Dongwoon Hyun, Stephen Rosenzweig, Gregg E. Trahey

Duke University

Purpose Short-lag spatial coherence (SLSC) imaging is an ultrasound beamforming method which forms images using the coherence of backscattered echoes. SLSC has been shown to significantly improve image quality over conventional B-mode, particularly in difficult-to-image patients exhibiting high levels of clutter. Previous studies have demonstrated improved visualization of a variety of clinically relevant structures; however, such studies have been limited to analyses of single images. Given the real-time nature of ultrasonic imaging, clinical translation of SLSC requires evaluation of its temporal stability and real-time performance. This study extends previous work to implement real-time SLSC imaging on the Siemens SC2000 clinical scanner. Development of this framework aims to provide a means to assess the feasibility and clinical utility of real-time ultrasound coherence imaging. **Methods** An SC2000 scanner is modified to acquire channel data and bypass internal processing for software beamformation. Due to bandwidth limitations, channel data is collected using synthetic receive sequencing, in which transmit firings are repeated to sequentially acquire echo data from each channel. To satisfy real-time requirements, SC2000 parallel receive capabilities are leveraged to acquire multiple channels in parallel. Subaperture beamforming in hardware is furthermore used to reduce channel data throughput. Images are beamformed on a pixel-by-pixel basis via GPU processing. Average spatial correlations between closely spaced channels are calculated to form each pixel in SLSC. B-mode pixels are found by taking the log-compressed magnitude of summed channel IQ data. Following beamformation, images are routed from the GPU to the scanner back-end for display. **Results** Real-time SLSC was successfully implemented with the Siemens 6C1 curvilinear array. Using this framework, channel data can be acquired with 3.5 MHz sampling and 16:1 parallel receive. B-mode and SLSC images (171 lines at 9 cm depth) are processed and displayed with frame rates ranging from 10 to 38 fps for subapertures of 3 to 12 elements respectively. Acquisitions from phantom and in vivo fetal scans demonstrate the system's real-time capabilities. In low SNR conditions, videos show improved contrast and CNR in SLSC over B-mode, consistent with previous findings. **Conclusion** Real-time coherence imaging was implemented on a clinical ultrasound scanner. Channel acquisition and GPU beamforming enable real-time display of B-mode and SLSC images at frame rates of up to 38 fps. Ultimately, clinical evaluation of such a system will provide valuable insight into the feasibility and utility of real-time SLSC imaging.

61. Cell density organizes collective migration through changes in actomyosin contractility

Andrew J. Loza, Sarita Koride, Gregory V. Schimizzi, Bo Li et al

Washington University in St. Louis School of Medicine

Purpose Collective migration of cells underlies embryonic development, tissue regeneration, and tumor invasion. Despite this widespread importance, there is still an incomplete understanding of the physical and biological mechanisms that allow multiple cells to organize their motion and furthermore, how these properties shape the specific types of collective migration that emerge. Using iterative hypothesis testing between simulation and experiment, we track the motion of thousands of cells to determine how the fundamental properties of cell density, adhesion, and actomyosin contractility combine to produce collective migration. **Methods** In this project we combine mathematical simulation, cell culture, and in-vivo approaches. Time-lapse imaging was performed on large regions of confluent MCF10A monolayers spanning a range of cell densities. Stable cell lines with altered adhesion and contractility were developed using lentiviral transduction. Simulations were carried out using a vertex model of cell migration. In vivo tests were performed on the developing drosophila follicular epithelium through 3D confocal time-lapse microscopy. **Results** We find that adhesion competent cells undergo an initial paradoxical decrease in organization with increasing density followed by an increase in organization at the highest densities. This trend reflects two processes. First, the high degree of organization at low density is produced by regulated actomyosin contractility. Enhancing or diminishing actomyosin contractility or uncoupling cells through by reducing cell-cell adhesion reduces organization. Second, the organization that appears at high density arises through adhesion and contractility independent mechanisms. Furthermore these two mechanisms of organization produce collectives with distinct patterns. Contractility mediated collective migration produces broad collective groups with cells moving in line with neighbors to either side. Packing mediated collective migration results in streaming patterns with cells following those in front and sliding past neighbors on either side. We test these predictions in-vivo using the drosophila follicular epithelium, a population of cells that undergoes a highly organized motion during egg development, providing insights into the initiation and maintenance of motion during this process. **Conclusions** This work demonstrates how commonly altered cellular properties can prime groups of cells to adopt migration patterns that may be harnessed in health or exploited in disease. High cell density and low cell-cell adhesion, as might be seen in cancer, transforms groups from broad collectives to small narrow streams potentially increasing invasive ability. Diminishing cell density, as might occur at a wound front, leads to large broad collectives with a distinct leader-follower structure that may accelerate wound healing.

62. Bioactivity and Adipogenic Potential of a Composite Adipose-Derived Hydrogel Scaffold for Soft Tissue Reconstruction

Christopher Mahoney, MS, Malik Snowden, J. Peter Rubin, MD, FACS, Kacey G. Marra, PhD
University of Pittsburgh

Introduction: Soft tissue reconstruction for the repair of congenital deformities or defects from tumor resections/trauma often require adequate replacement of adipose tissue. Standards of care include vascularized flaps or prosthetic implants consisting of silicone or saline. Although tissue flaps can have favorable results, complications may lead to flap failure, infections, pulmonary embolisms, and morbidity of the donor site. Autologous fat grafting using lipoaspirate is minimally invasive in reconstructive surgery but results are unpredictable due to resorption up to 10% volume retention. These limitations serve as motivation for developing therapies to regenerate adipose tissue within the tissue engineering field. **Materials and Methods:** Abdomen whole fat was donated from a non-diabetic female (age: 41, BMI: 26.3) undergoing elective cosmetic surgery at the University of Pittsburgh Medical Center. The decellularization process includes four main phases consisting of alcohol rinses, delipidization, and disinfection of the adipose matrix. After processing, the matrix was snap frozen using liquid nitrogen and then lyophilized. A Mini Wiley Mill breaks down the lyophilized matrix into a powder for pepsin digest and hydrogel formation. PLGA (50:50) was used as the base polymer to encapsulate fluorescent dexamethasone in microspheres using a single emulsion mixing technique. Adipose-derived stems cells (ASCs) were acquired using an isolation protocol on abdominal fat donated from a non-diabetic female (age: 38, BMI: 24.8) undergoing elective cosmetic surgery. Adipocyte quantification of ASCs study was conducted in a 12-well tissue culture plate along with Transwell tissue culture inserts to suspend the composite hydrogel above the cells in culture medium. The following culture conditions were used for comparison: cells with adipogenic medium, cells + composite hydrogel with adipogenic medium, cells + composite hydrogel without adipogenic medium (maintenance medium), and cells in maintenance medium (n=3). Cells were kept incubated at 37 °C and 5% CO₂. At day 7 and 14, mature adipocytes were stained using the AdipoRed™ Reagent Assay. **Results and Discussion:** Immunohistochemistry of matrix sections show that endogenous proteins were retained after the decellularization process. SEM images of the lyophilized hydrogel indicates porosity throughout the structure. As expected, higher concentrations of MS in hydrogel displayed a lower presence of porosity which may generate challenges for progenitor adipocytes migration into the scaffold. The 14-day differentiation study demonstrated higher amounts of adipogenesis in groups containing hydrogel and hydrogel with microspheres when compared to the positive control of ASCs alone. It is also important to note that adding the hydrogel to ASCs in maintenance medium resulted increased differentiation compared to the ASC in maintenance medium. **Conclusion:** The objective of this research project is to develop a composite hydrogel scaffold from discarded human adipose tissue for an enhanced adipogenic effect in the form of microspheres containing dexamethasone for the application of soft tissue engineering. The decellularization process has been found to be a reproducible process with consistent material production. These bioactivity studies allowed for further characterization of the

composite hydrogel. A 14-day differentiation study confirmed the scaffold's potential to increase proliferation and differentiation of adipose stem cells into adipocytes. Overall, the results warrant further investigation of the composite and its possible use in the regeneration of soft tissue.

63. Research maps: A semantic framework for causal discovery and experiment planning

Nicholas J. Matiasz, William Hsu, Alcino J. Silva, Wei Wang et al

UCLA

Purpose Biomedical researchers perform experiments to identify causal mechanisms. Their success depends on the quality of the experiments' designs and the results' interpretations. However, the model space for causal mechanisms is so large that it is unreasonable to expect researchers to consider all possible causal interpretations of the evidence in the literature. A causal system with only seven variables — for example, one showing how EGFR mutation affects contrast enhancement in T1-weighted MRI of gliomas — can be represented by over one billion possible causal graphs. To explore this vast model space exhaustively, researchers need the computational power of machines; however, as long as researchers remain “in the loop” of the scientific method, they also need to explore this model space using a framework whose semantics and epistemics they recognize. **Methods** Using a distilled taxonomy of experiments and evidence, we operationalized the strategies biologists use to identify causal mechanisms and expressed these strategies as the research map framework. A research map is a graphical representation of causal assertions and the evidence for these assertions; it integrates findings from multiple studies according to empirical principles in science: consistency and convergence. We have started to develop a complementary medicine map framework tailored to evidence in clinical trial articles. Whereas research maps can inform basic scientists' experiment planning, medicine maps can inform physicians' treatment of patients. We are also exploring how these representations can be used to specify constraints on traditional causal models (e.g., causal graphs). We are using state-of-the-art satisfiability solvers to process these constraints to find all causal interpretations of available evidence, thus informing the process of experiment planning. **Results** We implemented this framework in ResearchMaps, a web application with over 400 registered users across four continents. ResearchMaps allows users to create and visualize research maps for published articles, as well as query an integrated global map of all entries in the database. We have also started to develop MedicineMaps, which implements the complementary medicine map framework. The growing databases of these applications are providing data with which to evaluate our constraint-based experiment-planning approach. **Conclusion** Work in causal discovery is yielding robust formalisms for modeling causality. Our frameworks offer semantically rich representations for encoding causal information that can be used to plan experiments and define traditional causal models. These “meta-scientific” tools can facilitate the scientific method by guiding biologists' efforts to obtain new evidence while formalizing the evidence already published.

64. Design and Mechanical Testing of a Novel Magnesium Based Suture Anchor for Soft Tissue Fixation

Jonquil R. Mau, Kwang E. Kim, Antonio Pastrone, Dhir Patwa et al

University of Pittsburgh

Purpose Metallic and polymeric suture anchors have been used successfully for soft tissue fixation to bone (i.e. rotator cuff repair). However, there are advantages and disadvantages of each of these biomaterials. Titanium anchors could migrate and loosen as well as interfere with magnetic resonance imaging. Complications with polymeric suture anchors include osteolysis and breakage during insertion. Thus, we aim to develop a metallic suture anchor using Mg-based alloys, which is biodegradable, has the desirable mechanical properties and could promote bone remodeling. **Methods** The finite element method was used to determine the optimal threading for a Mg-based suture anchor (6.5 mm x 16.5 mm). A parametric analysis including thread pitch of 2.0 mm, 2.5 mm, 3.0 mm and thread depth of 0.4 mm, 0.7 mm, and 1.0 mm was conducted. A simulation of suture anchor pullout from polyurethane foam was conducted for each combination of design parameters. The design combination with the lowest von Mises stress was selected as the optimal design and manufactured. Then, the suture anchor underwent experimental pullout from polyurethane foam by an applied uniaxial load and the ultimate load and ultimate elongation were recorded. Polymeric suture anchor served as the control. **Results** From the parametric analysis, the thread pitch of 3.0 mm and thread depth of 1.0 mm were found to be optimal. From the mechanical test, the stiffness, ultimate load, and ultimate elongation were found to be 185 ± 13 N/mm, 379 ± 34 N, and 2.4 ± 0.2 mm, respectively for the Mg-based suture anchor and 107 ± 13 N/mm, 210 ± 13 N, and 1.9 ± 0.2 mm, respectively for the polymer suture anchor. The stiffness, ultimate load, and ultimate elongation were significantly different between the Mg-based and polymeric suture anchors ($p < 0.05$). **Conclusion** With these promising results, we believe it may be a superior alternative and are conducting an in-vivo animal study to evaluate its performance.

65. ImmunoPET engineering design considerations for imaging cancer immunotherapies

Aaron T. Mayer, Arutselvan Natarajan, Sydney R. Gordon, Roy L. Maute et al
Stanford University

Cancers have evolved to upregulate tolerogenic immune checkpoints, such as PD-L1, to suppress and evade the immune system. Although therapeutic antibodies targeted to block these immunosuppressive signaling pathways have exhibited remarkable success, many patients still do not respond to treatment. Imaging scientists are racing to validate biomarkers and develop tools to enable clinical response prediction, patient stratification and therapeutic monitoring of cancer immunotherapies. ImmunoPET offers the potential means to noninvasively assess dynamic immune checkpoint expression and the complex pharmacokinetics of antibody based drugs. Unfortunately, antibodies as imaging agents pose unique challenges including long blood circulation half-lives and high non-specific background. The engineering of small, high-affinity protein binders can potentially overcome these limitations and provide an accurate means to assess biomarkers for clinical checkpoint blockade. We have developed high affinity consensus (HAC)-PD1 [size = 14 kDa; $K_D = 100\text{pM}$], the first engineered binder to be employed for human PD-L1 immune checkpoint imaging. Here we sought to optimize ImmunoPET imaging of human PD-L1 expression, in a pre-clinical model, and assess the impacts of common radiotracer design parameters including chelate, glycosylation, and radiometal on tumor specific uptake and biodistribution. Five HAC-PD1 radiotracer variants were developed and assessed by small animal PET/CT studies. NSG mice were inoculated with subcutaneous tumors engineered to either be constitutively positive (CT26 hPD-L1) or negative ($\Delta\text{mPD-L1 CT26}$) for human PD-L1 expression and imaged with 20-60 μCi ($\sim 10\ \mu\text{g}$) of each HAC-PD1 radiotracer variant (3-6 mice/group). Of the design parameters tested, aglycosylating the radiotracer resulted in the greatest improvement in image quality showing significantly reduced nonspecific signal. The Cu64 variants accurately visualized PD-L1 expression, with Cu64-NOTA-HACA-PD1 exhibiting the highest tumor specific uptake [hPD-L1+ tumor: $2.3\pm 0.1\ \%\text{ID/g}$; hPD-L1- tumor: $0.9\pm 0.3\ \%\text{ID/g}$] and lowest background. Ga68 variants, which are more amenable to widespread clinical access, showed favorable biodistribution profiles, including Ga68-DOTA-HACA-PD1 which had the highest observed target tumor to background ratios for muscle [15.2x], lungs [6.3x], spleen [52x], pancreas [7.8x], small intestine [9.2x], large intestine [5.2x], bone [6.8x], and brain [68.2x]. The tracers primarily underwent renal clearance. Notably, all HAC-PD1 radiotracer variants enabled much earlier detection of human PD-L1 expression (1h post injection) than previously reported radiolabeled antibodies (> 24h post injection). This work provides a template for assessing ImmunoPET tracer design parameters and strongly supports the translation of small engineered protein radiotracers for imaging human immune checkpoints.

66. Simple, Scalable Proteomic Imaging for High-Dimensional Profiling of Intact Systems

Evan Murray, Jae Hun Cho, Daniel Goodwin, Taeyun Ku et al

MIT

Measuring diverse molecular and structural traits over multiple length scales remains a major challenge in biology. For decades, two-dimensional molecular phenotyping techniques have been utilized for investigating tissue samples. These techniques ensure similar reaction conditions by sectioning and limiting the length scale through which reactive molecules—such as fixatives, molecular probes, and antibodies—need to diffuse. Clearing techniques such as CLARITY are able to preserve the three-dimensional spatial arrangement of endogenous molecules and enable fluorescence imaging of intact biological systems (Chung et al., Nature 2013). However, slow diffusion of reactive molecules and molecular probes over system-wide length scales can cause uneven fixation and staining, respectively. Here, we introduce a simple method for the scalable, high-dimensional phenotyping of animal tissues and human clinical samples. This method, termed SWITCH, synchronizes tissue fixation across the entire system to uniformly secure the tissue architecture and native biomolecules. The preserved samples are robust to heat and chemical treatment and can be subjected to multiple rounds (>20) of relabeling. We have performed 22 rounds of labeling of a single tissue in combination with precise image co-registration. By attenuating reaction kinetics, SWITCH can also be applied to labeling reactions to improve probe penetration depth and overall staining uniformity. With SWITCH, we performed combinatorial protein expression profiling in the human cortex as well as examined the geometric structure of fiber pathways within mouse brains. SWITCH enables the extraction of high-dimensional protein expression information and may expedite our understanding of biological systems over multiple levels.

67. TOWARDS MULTIPLEX PROFILING AND CONTRAST ENHANCED RADIONUCLIDE IMAGING OF BREAST CANCER

Michael A. McDonald, M.D., Ph.D., Benjamin M.W. Tsui, Ph.D., Jingyan Xu, , Ph.D.

Johns Hopkins Medical Institutions

PURPOSE Multiplex profiling of the molecular needs of breast cancer (metabolism, perfusion, angiogenesis, hypoxia, pH, apoptosis, cell proliferation, receptor status, and signaling pathways) can facilitate early cancer detection and improve specificity relative to the current practice of imaging with single nonspecific agents. Radionuclide multiplexing has potential for early adoption and can bridge recent achievements in metabolomics/proteomics with precision medicine. The combination of energy-resolved image processing methods with single photon counting detector technology can facilitate multiplexing and also permit contrast-enhanced radionuclide imaging, potentially leading to both decreased radiation dose and decreased contrast agent dose. Our current research involves 1) multiplexing radionuclides at ultralow radiation dose and 2) development of contrast-enhanced radionuclide imaging methods, including Compton scatter-based imaging and dynamic contrast enhancement of scatter, for improved sensitivity of breast cancer detection. **METHODS** Sequential and/or simultaneous Tc99m, In111, and I123 phantom imaging studies were conducted in small animal- and clinical SPECT scanners. Energy spectra and contrast-enhanced images were acquired demonstrating attenuation of I123 by iodine- and gadolinium-based CT and MRI contrast agents as well as by Fe, Pd, Ag and Bi particulates. Simulated nuclear mammograms from a 4-D anthropomorphic breast phantom were acquired on NaI(Tl) scintillator (Dilon 6800; Dilon Technologies, Newport News, VA) and CZT solid-state detector (LumaGEM 3200S; Gamma Medica, Northridge, CA) modeled commercial breast imaging systems using analytical and Monte Carlo methods (GATE, SimSet). **RESULTS** Multiplexing of radionuclides using scintillation-based detectors is shown to be degraded by poor energy resolution and downscatter. Contrast agent detection at ultralow dose (50 uCi I123) through breast tissue equivalent material is demonstrated in a clinical SPECT scanner. Subtraction imaging shows potential for imaging at sub-mM contrast agent concentration. Similar analysis using simulated CZT-based detectors with higher energy resolution show significant improvement in resolving multiple energy peaks for Tc99m, In111 and I123. Subtraction images acquired before, during and after contrast agent administration demonstrate the possibility of tumor localization using dynamic contrast and scatter enhancement techniques. **CONCLUSION** Preliminary results demonstrate the advantages of utilizing energy discriminating detectors for radionuclide multiplexing and contrast/scatter enhancement including dynamic enhancement. Simulated breast tumor imaging is being used for the development and validation of novel dual- and triple energy constrained expectation maximization based image reconstruction techniques. On-going studies involve further improvement in sensitivity and specificity of breast tumor detection via development of a novel dual-modality breast imaging device incorporating CZT-based single photon counting detector technology for co-registered X-ray spectral- and molecular tomography.

68. Value of intra-tumoral metabolic heterogeneity and quantitative 18F-FDG PET/CT parameters to predict prognosis, in patients with HPV-positive primary oropharyngeal squamous cell carcinoma

Esther Mena, Mehdi Taghipour, Sara Sheikhabaei, Abhinav K. Jha et al

Johns Hopkins

Objective: To evaluate the impact of intra-tumoral metabolic heterogeneity and quantitative FDG-PET imaging parameters for predicting patient outcomes in primary oropharyngeal squamous cell cancer (OPSCC). **METHODS:** This is an IRB-approved, HIPPA-compliant retrospective study investigating 105 patients (mean age 58 ± 9.7 yo) HPV-positive OPSCC. Maximum and peak standardized uptake value (SUVmax, SUVpeak), total metabolic tumor volume (MTV), and total lesion glycolysis (TLG) were measured for each primary head and neck tumor and when available for metastatic lymph nodes and distant sites. Intra-tumoral metabolic heterogeneity of the primary tumor was calculated as the area under a cumulative SUV-volume Histograms curve (AUC-CSH). The median follow-up time was 35.4 months (range 3-92 months). Outcome endpoint was event free survival (EFS), including recurrence-free and overall survival. Kaplan–Meier survival plots and Cox regression analyses were performed. **RESULTS:** Of the 105 patients included in the study, 19 patients relapsed and 11 of them deceased during the study period. Univariate analysis demonstrated that optimum SUVmax ($p=0.006$; HR=5.8, 95%CI: 1.6-20.5), SUVpeak ($p=0.025$; HR: 3.3, 95%CI: 1.1-9.4), total MTV ($p=0.004$; HR= 3.1, 95%CI: 1.1-9.0) and total TLG ($p=0.033$; HR= 2.9, 95%CI: 1.1-7.7) were associated with EFS, and remained significant in multivariate analysis. AUC-CSH indexes were associated with EFS using either PET gradient-based ($p=0.034$) and 50%-Threshold ($p=0.022$) segmentation methods, on multivariate analysis. Kaplan–Meier survival analysis using optimum cut point of 16.7 SUVmax and 14.8 ml for total MTV were significant predictors of EFS. By stratifying SUVmax and AUC-CSH index in three subgroups, patients with higher degree of intratumoral heterogeneity and higher SUVmax values were associated with worse outcome (log-rank $p=0.026$). Similarly, patients with higher intra-tumoral heterogeneity tumors and higher MTV values had worse prognosis (log-rank, $p= 0.022$). **CONCLUSION:** Quantitative FDG-PET parameters, such as SUVmax, and MTV, and AUC-CHS indexes, as well as an integrated subgroup stratification of FDG avidity, total tumor burden and intra-tumoral heterogeneity appear to provide prognostic survival information for patients with primary OPSCC patients.

69. Quantitative breast MRI radiomics for cancer risk assessment and the monitoring of high-risk populations

Kayla R. Mendel, Hui Li, Maryellen L. Giger

University of Chicago

Breast density is routinely assessed qualitatively in screening mammography. However, it is challenging to quantitatively determine a 3D density from a 2D image such as a mammogram. Furthermore, dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is used more frequently in the screening of high-risk populations. Purpose: The purpose of our study is to segment parenchyma and to quantitatively determine volumetric breast density on pre-contrast axial DCE-MRI images (i.e., non-contrast) using a semi-automated quantitative approach. Methods: In this study, we retroactively examined 3D DCE-MRI images taken for breast cancer screening of a high-risk population. We analyzed 66 cases with ages between 28 and 76 (mean 48.8, standard deviation 10.8). DCE-MRIs were obtained on a Philips 3.0 T scanner. Our semi-automated DCE-MRI algorithm includes: (a) segmentation of breast tissue from non-breast tissue using fuzzy c-means clustering (b) separation of dense and fatty tissues using Otsu's method, and (c) calculation of volumetric density as the ratio of dense voxels to total breast voxels. Results: We examined the relationship between pre-contrast DCE-MRI density and clinical BI-RADS density obtained from radiology reports, and obtained a statistically significant correlation [Spearman ρ -value of 0.66 ($p < 0.0001$)]. Conclusion: We observed a significant correlation between pre-contrast DCE-MRI density and radiologist-assigned BI-RADS density. Therefore, we believe that pre-contrast DCE-MRI breast segmentation could be useful in future feature assessment, such as texture analysis and DCE contrast enhancement assessment. We conclude that pre-contrast DCE-MRI images can be used to obtain a clinically relevant measure of breast density. Our method within precision medicine may be useful for monitoring high-risk populations.

70. Influence of Cardiovascular Risk Factors in Brain Networks and Dementia.

Manuel Morales, Curtis Thorne, Steven Altschuler, Lani Wu

Harvard-MIT Health Sciences & Technology

Purpose: It is important to understand the preventable and treatable causes of dementia. Evidence is accumulating to suggest that cardiovascular disease (CVD) risk factors may be instrumental in the development of dementia. The objective of the present study is to elucidate the relationship between specific CVD risk factors and dementia. Two approaches will be taken to investigate the relationship between CVD risk factors and dementia: (1) examine the effects of CVD on resting-state fMRI brain networks in patients with mild cognitive impairment (MCI); and (2) identify the relationship between microinfarcts and MCI by assessing the possible pathophysiological causes behind microinfarcts.

Methods: To validate our network modelling approach, network modelling of resting-state fMRI time series data was conducted in $\epsilon 4$ variant of the APOE allele associated with Alzheimer's disease, as well as non-carriers, using independent component analysis (ICA) to categorize the various resting-state networks and identify population differences. Future cohorts will include MCI patients with and without cardiovascular risk factors.

Results: Full correlation resting-state network matrices were calculated using ICA network modelling. The edge strengths of these network matrices were used in a machine learning algorithm and group differences were found. Interpretation of group differences was problematic due to the high sensitivity of the network matrices to head motion, cardiac and breathing cycles, and scanner artefacts.

Conclusions: Motion correction methods of head motion, cardiac and breathing cycles will have to be developed to improve the performance of the ICA network modelling.

71. Image Reconstruction Techniques for a Portable Head-only 1.5 T MRI System

Michael Mullen, Jinjin Zhang, Albert Jang, Djaudat Idiyatullin et al

University of Minnesota

Purpose: To develop a magnetic resonance imaging (MRI) technique and an image reconstruction algorithm for studying the human brain with a small, head-only magnet that produces a non-uniform magnetic field. The MRI signals to be reconstructed are obtained using either: 1) a spatiotemporally-dependent RF pulse (see [1]), or 2) a conventional RF pulse, but with an array of coils driving a “shimmed” volume over the brain. **Methods** To simulate the behavior of magnetization for various pulses and magnetic field topologies, an empirical description known as the Bloch equations were used. What is known as the encoding matrix, which describes the magnetization at a given point over time, is numerically determined in this manner. The signal is then related to a summation over this encoding matrix. To overcome complications arising from the non-uniform magnetic field, the two aforementioned methods of obtaining a signal were used. In case 1, the RF pulse compensates for the magnetic field inhomogeneity, while in case 2, the shimmed volume possesses a nearly uniform magnetic field due to coil array. One method of reconstruction is the Maximum Likelihood Estimation (MLE), which compared the signal corresponding to an approximate image to that of the simulated signal. A likelihood function is defined in such a way as to be maximized when the difference between the two goes to zero. Another method of reconstruction took advantage of the sparsity of the encoding matrix in the spatial Fourier space. **Results** For both methods of inhomogeneity compensation in a small magnet, the reconstruction techniques discussed successfully constructed images, showing the viability of image acquisition in such an MRI technique. The latter reconstruction algorithm proved to be more reliable in terms of efficiency and accuracy. Note these results have only been verified on simulation results and phantoms, not yet on true brain scans. However, the results up to this point are promising and are indicative that this approach should work in this regime, as well. **Conclusions** In summary, this project moves forward in demonstrating the feasibility of a portable, head-only MRI system which can produce reliable brain images. The ability to perform small-scale MRI will open exciting new possibilities for neuroscientists to study the brain and human behavior in a diverse range of conditions. **References** [1] Jang, A., Kobayashi, N., Moeller, S., Vaughan, J. T., Zhang, J. and Garwood, M. (2015), 2D Pulses using spatially dependent frequency sweeping. *Magn Reson Med*. doi: 10.1002/mrm.25973 Funding NIH 2T32 EB008389, NIH R24MH105998, and NIH P41EB015894

72. Tracking human neural progenitor cells derived from pluripotent stem cells using mitochondrial ferritin as an MRI reporter gene

Kazim H Narsinh, Shang Gao, Hongyan Xu, Martin Marsala et al

UC San Diego

Introduction An unmet challenge to successful translation of stem cell therapies into patients is the ability to non-invasively monitor cellular behavior and movement following transplantation. Imaging tools can potentially provide critical information regarding the homing, engraftment, and proliferation of the delivered cells. MRI can track cells by direct labeling with iron-oxide nanoparticles or gadolinium chelates, but these agents become diluted after mitotic cellular divisions, thereby limiting long-term visualization, and may fail to discriminate between living versus dead cells. Genetically-engineered ferritin constructs can be used as MRI reporter genes to overcome these limitations (1). Upon cellular expression, ferritin forms a superparamagnetic iron core that generates hypointensity in T2- and T2*-weighted images. Because the ferritin gene can be stably passed to daughter cells, long-term tracking studies of cellular therapeutics may be performed. Here, we report the use of a modified mitochondrial ferritin construct, with improved MRI sensitivity over wild-type ferritins, to monitor the engraftment and survival of human neural progenitor cells after their transplantation into rat striatum.

Materials and Methods We inserted an engineered human mitochondrial ferritin gene (2) into a lentiviral construct already containing green fluorescent protein (GFP) under a human ubiquitin promoter. We then transduced human embryonic stem cell-derived neural progenitor cells (hESC-NPCs) with the lentiviral construct to generate hESC-NPC lines that stably expressed mtFt. Twenty adult male athymic nude rats underwent stereotactic implantation of mtFt-expressing hESC-NPCs into their striata, while nontransduced hESC-NPCs were stereotactically implanted into the contralateral striata as a control. On day 7, 14, 28, 90, and 180 after implantation, *in vivo* MRI was performed at 11.7 T using a 3D gradient-recalled echo (GRE) sequence with TE/TR=7/100 ms and 117 μ m isotropic voxels. *Ex vivo* imaging at 11.7 T was also performed. Immunohistochemistry was performed on the fixed rat brain tissue using rabbit polyclonal antibody against mtFt, mouse anti-nuclear protein/h-nuc antibody (anti-hNUMA) specific to a human antigen, and goat anti-GFP antibody.

Results and Discussion After injection into rat brain cortex, hESC-NPCs expressing mtFt were clearly detected as hypointense regions on T2*-weighted GRE images. The contralateral side demonstrated normal signal intensity. Immunohistochemical analysis confirmed that the hypointense regions contained transplanted cells expressing GFP. Truncated mtFt localized to the cytoplasm and loaded more iron than wild-type mtFt or human ferritin. The ferritin-expressing cells identified by MRI were confirmed as hESC-NPCs by immunohistochemical staining for a human specific nuclear antigen. This optimized MRI reporter gene has significant potential for long-term monitoring of the success of stem cell transplantation studies.

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73. A Comprehensive Formulation for Volumetric Modulated Arc Therapy Planning

Dan Nguyen, Qihui Lyu, Dan Ruan, Daniel O'Connor et al

UCLA Physics and Biology in Medicine Graduate Program

Purpose: Volumetric modulated arc therapy (VMAT) is a widely employed radiotherapy technique, showing comparable dosimetry to static beam intensity modulated radiation therapy (IMRT) with reduced monitor units and treatment time. However, the current VMAT optimization has various greedy heuristics employed for an empirical solution, which jeopardizes plan consistency and quality. We introduce a novel direct aperture optimization method for VMAT to overcome these limitations.

Methods: The comprehensive VMAT (comVMAT) planning was formulated as an optimization problem with an L2-norm fidelity term to penalize the difference between the optimized dose and the prescribed dose, as well as an anisotropic total variation term to promote piecewise continuity in the fluence maps. A level set function was used to describe the aperture shapes and the difference between aperture shapes at adjacent angles was penalized to control MLC motion range. A proximal-class solver was adopted to solve the large scale optimization problem, and an alternating minimization was implemented to solve the fluence and aperture shapes simultaneously. Single arc comVMAT plans, utilizing 180 beams with 2° angular resolution, were generated for a glioblastoma multiforme (GBM) case, a lung (LNG) case, and 2 head and neck cases—one with 3 PTVs (H&N3PTV) and one with 4 PTVs (H&N4PTV). The plans were compared against the clinical VMAT (clnVMAT) plans utilizing two overlapping coplanar arcs for treatment.

Results: The optimization of the comVMAT plans had converged within 600 iterations. comVMAT plans were able to consistently reduce the dose to all organs-at-risk (OARs) as compared to the clnVMAT plans. On average, comVMAT plans reduced the max and mean OAR dose by 6.59% and 7.45%, respectively, of the prescription dose. Reductions in max dose and mean dose were as high as 14.5 Gy in the LNG case and 15.3 Gy in the H&N3PTV case. PTV coverages measured by D95, D98, and D99 were within 0.25% of the prescription dose. By comprehensively optimizing all beams, the comVMAT optimizer gained the freedom to allow some select beams to deliver higher intensities, yielding a dose distribution that resembles a static beam IMRT plan with beam orientation optimization.

Conclusions: The novel non-greedy VMAT approach simultaneously optimizes all beams in an arc and then directly generates deliverable apertures. The single arc VMAT approach thus fully utilizes the digital linacs' capability in dose rate and gantry rotation speed modulation. In practice, the new single VMAT algorithm generates plans superior to existing VMAT algorithms utilizing two arcs.

74. Development of Glutamate-Sensitive, Chemical Exchange Saturation Transfer Imaging at 7 Tesla for Application to Multiple Sclerosis

Kristin P. O'Grady, Adrienne N. Dula, Bailey D. Lyttle, Benjamin N. Conrad et al
Vanderbilt University Institute of Imaging Science

Purpose: Glutamate is the principal excitatory neurotransmitter in the brain, and dysfunctional glutamate regulation is implicated in the pathogenesis of chronic neurodegenerative processes. In multiple sclerosis (MS), glutamate-mediated excitotoxicity leads to neuronal death, and magnetic resonance spectroscopy studies have detected altered glutamate metabolism in MS and linked gray matter glutamate levels with cognitive impairment. Pathology in gray matter is subtle and difficult to detect, and there is a need for the development of imaging biomarkers that will enable detection of early molecular changes, prediction of future cognitive impairment, and evaluation of treatment response. Recently, glutamate-sensitive chemical exchange saturation transfer (GluCEST) has been explored and we extend the application of GluCEST at 7T in the brain for healthy controls and MS patients. We hypothesize that the GluCEST signal will be altered in MS patients, and we examine correlations with measures of cognitive impairment. **Methods:** A 2D multi-shot TFE sequence (factor=3, 0.94 x 0.94 x 10 mm resolution) was applied in thirty-five healthy controls (ages 22-56) and 24 MS patients (ages 30-44) at 7T. CEST data were acquired using a 4.25 T pulse train of ten 10 ms RF pulses (total saturation duration = 100 ms) at 49 frequency offsets between -5.0 and 5.0 ppm and at one offset far off resonance (=80.0 ppm) for reference. A T1-weighted image was obtained for segmentation. CEST data were corrected for B1 inhomogeneity, and z-spectra were centered with a B0 correction derived from a Lorentzian fit. Several methodologies for quantifying GluCEST were compared, including $[M_{\text{sat}}(-) - M_{\text{sat}}(0)] / M_{\text{sat}}(-)$ computed at = 3.0 ppm, where an exchange effect between glutamate amine protons and bulk water has been reported. Correlations between GluCEST and cognitive function were investigated in MS patients. **Results and Conclusions:** Preliminary analysis of GluCEST data was performed on a subset of healthy (n=7) and MS (n=6) subjects (age range of 30-37). The mean GluCEST contrast for gray matter in each MS subject correlates significantly with the Global Deterioration Score (overview of cognitive function) with a Spearman's correlation coefficient of 0.912. In ongoing work, we will further optimize our approach for processing and quantifying the GluCEST data and will examine correlations with additional cognitive measures using our complete set of subjects. **Acknowledgments:** NIH/NIBIB K01EB009120, KL2 TR 000446, CTSA Grant (RR024975), DoD W81XWH-13-0073, NIH/NIBIB 5T32EB001628-14

75. Molecular Recognition of Spermine by LnDOTP5⁻: Toward a Noninvasive Staging of Prostate Cancer

Abiola O. Olatunde, Taylor L. Fuss, Philip Z. Sun, Leo L. Cheng et al

Massachusetts General Hospital

Purpose. Spermine is an important biomarker of prostate health and its concentration is inversely correlated with the presence of cancer. In vivo quantification of spermine by MR spectroscopy is limited because the chemical shift of the spermine protons (ca 1.8 ppm) overlap with signals from other metabolites in this region. LnDOTP5⁻, a stable anionic lanthanide (Ln(III)) macrocyclic complex, forms a sufficiently stable ternary complex with the positively charged spermine. The ion-pair interaction results in the selective shift of the spermine MR resonances with the magnitude and direction of the shift dependent on the pseudocontact contribution of the lanthanide. Here, we report the affinity of different LnDOTP5⁻ complexes for spermine and the effect of complex formation on spermine MR resonances in D2O, serum solutions and intact human prostate tissue. **Method.** Intact Tissue. Frozen tissue was scanned using high-resolution magic angle spinning (HRMAS) MRS on a Bruker AVANCE spectrometer operating at 600 MHz (14.1T). A 4 mm zirconia rotor with Kel-F inserts created a 10 µl sample space for tissue samples, and D2O was added for field locking. After an initial scan, 5 µl LnDOTP5⁻ was added to one rotor and 5 µl of D2O was added to another rotor as control. Both rotors were kept overnight at 4°C and then rescanned the next day. Spectra were recorded at 4°C with the spectrometer frequency set on the water resonance. Spectra were measured with HRMAS with a spin rate of 3600Hz (±1.0Hz), and analyzed using an in-house developed MatLab based program. **D2O and Serum Samples.** Prepared samples of 10 mM spermine and 10 mM citrate in D2O or serum were analyzed with MRS on the same Bruker spectrometer. D2O was added to serum samples for field locking. Eu(III), Yb(III), and Tm(III) complexes were evaluated at 4°C and 37°C. **Results and Conclusion.** Spermine forms stable 1:1 complexes with LnDOTP5⁻ ($K > 10^5$ M) and the lanthanide-induced shift can be large, with shifts up to 100 ppm for the spermine-TmDOTP5⁻. The results of experiments exploring the effects of ions, such as Zn(II), Ca(II), and phosphate, as well as the observed changes in citrate and lactate in tissue, has enhanced our understanding of the ion-pair interactions of spermine-LnDOTP5⁻ complex in physiological conditions. In the presence of competing anions (phosphate, lactate and citrate), the shift of spermine-LnDOTP5⁻ complex is reduced due to the spermine-metabolite interactions in serum and D2O. The ion-pair interaction provides a means for distinguishing metabolite-metabolite interactions in tissue.

76. Functional Network Reorganization of Multimodal Integration Regions in Blind Children

Laura Ortiz-Teran, Ibai Diez, Tomás Ortiz, David L. Perez et al

Massachusetts General Hospital

PURPOSE: Cross-modal neuroplasticity has been proposed as a mechanism by which individuals without sight recruit visual-related cortices to process sensory information from other perceptual modalities. We have previously observed in adults with blindness compared to healthy subjects that multimodal integration regions are prominent sites of neuroplastic reorganization. This study uses network-based functional connectivity analyses to investigate network connectivity differences in blind children compared to controls. We hypothesize that in addition to connectivity differences in visual and other sensory cortices, blind subjects show functional connectivity changes that centralize within multimodal integration regions. **MATERIAL AND METHODS:** We studied 13 children with blindness (N=9 boys) ages between 7-12 years old (mean=9.6±1.3), and 15 sighted controls (N=6 boys) (mean=10.3±1.4). Subjects were scanned on a 3T MRI scanner, acquiring BOLD and high-resolution 3D T1WI. Following pre-preprocessing, whole brain weighted-degree functional connectivity and step-wise connectivity graph theory analyses were applied. **RESULTS:** In weighted-degree analyses corrected for multiple comparisons, blind children exhibited enhanced connectivity in bilateral ventral premotor, middle cingulate cortex/supplementary motor area and right temporal parietal junction. Several of these connectivity changes positively correlated with age. Using step-wise connectivity analysis, blind children compared to controls demonstrated increased functional streams along certain multimodal integration regions such as the anterior insula and temporoparietal junction bilaterally and right lateral occipital cortex. **CONCLUSIONS:** Blind children show increased functional connectivity in multimodal integration areas compared to controls, and older children showed greater increases within these regions.

77. Clinically relevant factors affecting catheter motion in Intracardiac Echocardiography (ICE) Acoustic Radiation Force Impulse (ARFI) Imaging

Jenna K. Osborn, Young-Joong Kim, Stephanie Eyerly, Patrick D. Wolf

Duke University

Purpose: Acoustic Radiation Force Impulse (ARFI) Imaging is an ultrasound-based imaging technique that utilizes a high intensity acoustic wave to remotely displace tissue with a momentum transfer. The displacement of the tissue can be tracked using conventional ultrasound and can be used to visualize variations in tissue elastic properties. In intracardiac echocardiography (ICE) catheter ARFI imaging, momentum is also transferred to the catheter causing a kickback motion. The motion could potentially degrade the integrity of the induced displacement measurements as all are made relative to the transducer. To address these issues in a clinical setting, the relevant factors were examined. Methods: A 3 kPa homogeneous elastography phantom was imaged using an 8F SoundStar™ ICE catheter with custom ARFI imaging sequence (30 pushes, 15 mm focus, 6 MHz transmit frequency) on a Siemens SC2000™ scanner. All pushes were directed toward the center line and were temporally equivalent to current clinical sequences used in other protocols. The fulcrum length, the distance from a mechanically fixed point to the tip of the catheter, was varied from 30 mm to 50 mm. Different mechanical steering configurations in the same dimension as the kickback motion were tested for possible improvements. The kickback displacements were measured 10 mm from the focal point to isolate the kickback motion from the induced ARFI displacements. The sequential pushes were aligned in time to evaluate the accumulation of the kickback over the sequence. Results and Conclusions: For fulcrum lengths greater than 40 mm, mechanical oscillations were seen with different frequencies and amplitudes depending on the fulcrum length. The mechanical steering did not significantly improve the accumulated displacements over the angles tested. For the imaging parameters tested, kickback motion is 2-3 μm per push recovering to a net 0.4 μm per push by the end of tracking. This motion could be a source of bias in ICE catheter ARFI imaging and a solution is desired.

78. Kinetic Analysis of [18F](2S,4R)4-Fluoroglutamine In Mouse Models of Breast Cancer with Glutaminase Inhibition

Austin Pantel, Rong Zhou, Hsiaoju Lee, Shihong Li et al

Hospital of the University of Pennsylvania

PURPOSE: Alterations of cellular metabolism in malignancy represent opportunities for nuclear probe development. As an alternate nutrient source to glucose, tumors may utilize glutamine, the most abundant amino acid in blood. Inhibitors of glutamine metabolism represent potential therapeutic targets. Inhibitors of glutaminase, the enzyme responsible for the conversion of glutamine to glutamate, are in early clinical trials. [18F](2S,4R)4-Fluoroglutamine ([18F]Fluoroglutamine), an analog of glutamine for PET imaging, has been evaluated in pre-clinical studies. Based on cellular data, we hypothesize that tumors with elevated glutaminolysis (triple-negative breast cancer (TNBC) tumors), which have lower intracellular glutamine pool size, would have lower [18F]Fluoroglutamine distribution volumes compared to tumors with low glutaminase activity (MCF-7 tumors). Additionally, glutaminase inhibition would increase the distribution volume for [18F]Fluoroglutamine. **METHODS:** TNBC cells (HCC1806) were subcutaneously inoculated in the flank of athymic nu/nu mice. After adequate growth, imaging was performed on a dedicated small animal PET scanner. [18F]Fluoroglutamine was injected into the lateral tail vein at the start of dynamic image acquisition and images were obtained for 60 minutes at 5 minutes/frame. A glutaminase inhibitor was then administered per protocol while a control mouse received a saline-based vehicle solution. Mice were then reimaged using the same protocol. A mouse inoculated with MCF-7 cells was also imaged. Image analysis was performed using AMIDE data analysis software. Kinetic analysis was performed using PMOD. **RESULTS:** Logan plot analysis revealed linearity from which distribution volumes of [18F]Fluoroglutamine were estimated. Preliminary Logan plot analysis of the two TNBC tumor-bearing mice demonstrated an increased volume of distribution post-glutaminase inhibition with individual estimates of ~10% and nearly 50% change. No increase in volume of distribution was seen in the vehicle-treated TNBC mouse. The untreated mouse with the MCF-7 tumor demonstrated a volume of distribution at least 50% larger than the TNBC untreated tumors. **CONCLUSION:** Preliminary kinetic analysis identified increased volume of distribution of [18F]Fluoroglutamine post glutaminase inhibition in a mouse model of breast cancer with elevated glutaminase activity. Furthermore, the volume of distribution of this radiotracer was greater in tumors with low glutaminase activity compared to tumors with elevated glutaminase activity. Kinetic modeling of [18F]Fluoroglutamine represents a promising tool to measure the impact of glutaminase-directed therapy.

79. Silicon nanowires as a platform for wireless optical modulation of neuronal activity

Ramya Parameswaran, Joao L Carvalho-de-Souza, Ektor Acaron Ledesma, Michael J Burke et al
Biophysical Sciences University of Chicago

The development of minimally invasive methods to modulate electrical activity in cellular systems with high spatiotemporal resolution has been a significant challenge for many years now. Shapiro et al. and Carvalho-de-Souza et al. have recently demonstrated that IR light and gold nanoparticles can stimulate neurons photothermally. Here, we explore an inorganic platform that when interfaced with neurons, can modulate neural activity via a photovoltaic effect. We demonstrate that coaxial pin Silicon nanowires consist of both a radial p-n diode component and an axial Au-Si diode component caused by the diffusion of the gold nanoparticle catalyst along the nanowire side-walls during growth. In culturing these nanowires with primary rat dorsal root ganglion cells, we show that upon localized laser stimulation at the cell-nanowire interface, they can efficiently induce action potentials in individual neurons. These findings provide us with a novel method to optically modulate neuronal activity in a wireless manner and thus a potential therapeutic strategy for patients suffering from neurodegenerative diseases.

80. Spatial Response of Double-Sided Strip High-Purity Germanium Detectors for SPECT Imaging

Perea, Rose, Campbell, Desmond L., Shokouhi, Sepideh, Peterson, Todd E.

Physics and Astronomy at Vanderbilt University

Purpose: Single Photon Emission Computed Tomography (SPECT) is a nuclear medicine imaging technique that allows mapping of the biological distribution of an injected radiotracer. We are developing a dual-headed small-animal SPECT system using double-sided strip High-Purity Germanium (DSS HPGe) detectors. HPGe provides superior energy resolution (<1% FWHM at 140 keV), which allows for good scatter rejection and facilitates dual- or multi-isotope imaging. The electrode strip configuration allows sub-strip positioning of events. Reconstructed images in our prototype small-animal SPECT system exhibited artifacts, which we attributed to a combination of mis-positioning of events near the strip edges and loss of events (due to charge sharing and multiple-strip events). The objective of this study is to develop and deploy advanced signal processing techniques to enhance the performance of our DSS HPGe detectors, which will lead to improvements in the sensitivity, spatial resolution, and image contrast of our SPECT scanner. **Methods:** The detector used in this study consists of an HPGe crystal, 90 mm in diameter with 16 x 16 orthogonal strips. The strip widths are 4.75 mm with 0.25 mm gaps between strips. The depth of interaction is estimated from the arrival time differences of the signals on the two sides of the detector. The detector and readout were fabricated by PHDs Co in Knoxville, TN. To study the detector response and acquire data to develop methods to recover multi-strip events, we have scanned a detector with a focused beam ($\sim 25 \times 25 \mu\text{m}^2$ @ 131 keV) at the Advanced Photon Source (APS, Argonne National Lab). We obtained three different data types using the detector acquisition software: list-mode data (fully shaped, digitized, binned into sub-pixels, two-strip events only), raw data (fully shaped and digitized, not binned, all events), and waveform data. **Preliminary Results:** The preliminary data reveals efficiency losses that extend more than 100 micrometers beyond the gap region as well as variations in spatial resolution across the strip area using our current processing. From the waveform data, events show a clear signal on the collecting strip, and fast transient signals induced on neighboring strips. **Future Work:** Our next steps are to perform a full detector scan at APS to develop distortion and efficiency correction techniques for improvement to our current position estimation method, as well as explore advanced signal processing methods to further improve performance. Improvements in event estimation should remove artifacts and lead to better contrast and sensitivity in SPECT images.

81. Reversed Gradient-Spoiled Diffusion-Weighted Imaging in the Breast with PSIF

Stephanie L. Perkins, Bruce L. Daniel, Brian A. Hargreaves, Catherine J. Moran
Stanford University

Purpose: Breast MRI protocols including contrast-enhanced and non-contrast scans are routinely used to aid in the diagnosis of breast cancer. Diffusion-weighted imaging (DWI) is a non-contrast scan that may aid in distinguishing between benign and malignant breast lesions when used as an adjunct to contrast-enhanced breast MRI. PSIF is a reversed gradient-spoiled sequence with diffusion weighting that has been shown to increase SNR, improve spatial resolution, and reduce distortion in the breast relative to traditional DWI. However, known challenges of using PSIF in the breast include sensitivity to motion, fat-water separation, and decreased tissue-lesion contrast relative to DWI. The goal of this work was an initial attempt to overcome these challenges. **Methods:** Sagittal PSIF scans were performed in a fat-water phantom and in volunteers on a GE Discovery MR750 3T scanner. Imaging parameters were modified from typical DWI to fit scans within a breath-hold. The amount of spoiling applied across the voxel was changed between scans to vary the diffusion weighting. The amount of diffusion weighting was examined qualitatively by plotting the mean signal in fat, water, and lesion ROIs as a function of cycles of spoiling. The effects of k-space ordering (sequential vs. elliptical-centric) on motion artifacts that appear at higher cycles of spoiling were also analyzed. A radiologist with breast MRI experience also provided an initial assessment of the images to give feedback on the motion artifacts, fat-water separation, and tissue-lesion contrast. **Results:** In the phantom, the mean fat ROI signal remained constant as the cycles of spoiling was increased but the mean water ROI signal decreased. In vivo, motion artifacts at higher cycles of spoiling appeared to affect the fat-water separation, and thus the mean signal in the ROIs. Using elliptical-centric k-space ordering instead of sequential ordering removed motion artifacts seen in the phantom but not completely in vivo. The effect of cycles of spoiling on tissue-lesion contrast was not consistent across subjects in vivo. **Conclusion:** The phantom results of decreased water ROI signal with increased cycles of spoiling provides motivation for continued investigation, although parameter optimization taking into account motion artifacts and fat-water separation needs to be resolved. Future work will include analyzing the effect of applying cycles of spoiling in different directions on the motion artifacts and fat-water separation, and comparing image contrast between traditional DWI and PSIF scans to determine the cycles of spoiling necessary for adequate diffusion weighting.

82. A Biomimetic Platform Reveals Novel Mechanisms for Regulation of Microvascular Function via Hemodynamic Shear Stress

William J. Polacheck, Matthew L. Kutys, Christopher S. Chen

Harvard University

Regulation of vascular permeability is critical to cardiovascular function, and misregulation of vascular permeability contributes to a host of cardiovascular diseases. Hemodynamic shear stress is a key determinant of vascular homeostasis and known to be a critical regulator of vascular permeability. However, the molecular mechanisms that regulate vascular permeability in response to shear stress remain poorly understood due to the lack of experimental systems that recapitulate hemodynamically controlled blood flow through an endothelial lumen surrounded by 3D ECM. Here, we introduce a microvasculature-on-chip model that leverages the physical and biochemical control of in vitro systems and integrates 3D ECM and vessel architecture to investigate the effects of shear stress on vascular barrier function in a precisely regulated, physiologically relevant microenvironment. We further implement the platform and CRISPR-based gene editing in primary cells and cell lines to identify novel mechanisms that govern shear-regulated vascular barrier function.

83. High Resolution Steady State Blood Volume Maps in Glioblastoma Using MRI

Joao Prola Netto, Csanad Varallyay, Prakash Ambady, Jenny Firkins et al
Oregon Health and Science University

Purpose: Glioblastoma (GBM) is the most common infiltrative primary malignant brain tumor. Magnetic resonance imaging (MRI) using gadolinium based contrast agents (GBCAs) is routinely used in the diagnosis and evaluation of therapeutic response. DSC perfusion MRI has been tested in multiple studies, and its value in brain tumors has been shown, however due to certain limitations it is not used widely in the clinical practice. Steady state (SS) imaging using ferumoxytol tries to overcome these limitations providing high resolution cerebral blood volume (CBV) maps. The primary objective of the study will compare SS-CBV with DSC-CBV parametric maps using visualization variables. Secondary objectives of the study are correlation with histology and immunohistology, association of CBV with overall survival, differentiation of progression from pseudoprogression, and assessment of late ferumoxytol enhancement. **Methods:** This prospective imaging study was approved by the local institutional review board. Subjects with suspected or confirmed diagnosis of GBM are eligible. The subjects undergo 3 consecutive days of MRI scans; first day with and without gadolinium, second day with and without ferumoxytol, and a third day with no contrast to study late ferumoxytol uptake. Research scans are done at specific time points at various stages of disease. After acquisition, patient data is de-identified and post processing and analyses are done in a dedicated workstation. Comparison between SS-CBV with DSC-CBV maps will be done using four visualization variables each using a 3 point scale. **Results:** 9 patients have been enrolled with 18 time points of scans: 5 before surgery; 5 after surgery and before chemoradiation therapy (CRT), 5 after CRT; and 3 by the time of recurrence. 5 patients are still being followed. In all the patients the SS CBV maps were superior to DSC CBV maps in the visualization criteria (assessment of overlay accuracy with T1w post contrast scans; confidence in identifying the lesion corresponding areas on CBV maps; assessment of CBV in small lesions; and separation of tumor from larger blood vessels.) **Conclusion:** This preliminary data shows the feasibility of the SS CBV maps with ferumoxytol in patients with GBM. Initial analyses of the obtained maps showed superiority of SS CBV maps when compared to DSC CBV maps.

84. Preventive Neuroradiology in Brain Aging and Cognitive Decline

Cyrus A. Raji, MD, PhD,

UCSF Department of Radiology

Preventive neuroradiology is a new concept supported by growing literature. The main rationale of preventive neuroradiology is the application of multimodal brain imaging toward early and subclinical detection of brain disease and subsequent preventive actions through identification of modifiable risk factors. An insightful example of this is in the area of age-related cognitive decline, mild cognitive impairment, and dementia with potentially modifiable risk factors such as obesity, diet, sleep, hypertension, diabetes, depression, supplementation, smoking, and physical activity. In studying this link between lifestyle and cognitive decline, brain imaging markers may be instrumental as quantitative measures or even indicators of early disease. The purpose of this article is to provide an overview of the major studies reflecting how lifestyle factors affect the brain and cognition aging. In this hot topics review, we will specifically focus on obesity and physical activity.

85. Evaluating force representation in motor cortex of an intracortical BCI user with chronic tetraplegia

Anisha Rastogi, Brian A. Murphy, Frank R. Willett, William D. Memberg et al
Case Western Reserve University, Department of Biomedical Engineering

Background: Intracortical brain computer interfaces (iBCIs) have emerged as a promising assistive technology for restoring hand grasping in individuals with tetraplegia. To date, most iBCIs intended for human use have utilized only kinematic information from the motor cortex. However, during natural hand grasping, the motor cortex encodes a combination of kinematic and kinetic information. Previous studies in nonhuman primates have investigated the feasibility of utilizing kinetic neural information, identified during executed force production, as control signals for iBCIs. Here, we further elucidate how force-related information is represented in the motor cortex in an individual with chronic tetraplegia. Specifically, we characterize the extent of neural modulation that occurs during observed, imagined, and attempted forces. **Methods:** Participant T8 of the BrainGate2 Clinical Trial (55-year-old male, C4-level spinal cord injury) was asked to observe, imagine, and attempt producing four discrete force levels with the dominant hand. Full broadband neural recordings were obtained from two 96-channel microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT) in the dominant precentral gyrus. We characterized the modulation of two time-varying features (spike firing rates, high frequency spike powers) during force production. These features were also used as inputs to a linear discriminant analysis (LDA) classifier, to discriminate the observed, imagined, and attempted force levels offline. **Results & Conclusions:** The number of neural features tuned to force production, as well as offline discrimination performance, was greatest during attempted force, and least pronounced when force production was observed. Additionally, tuned features exhibited various temporal profiles, with some tuned to the preparatory phase of force production, others tuned to active force production, and still others tuned to both phases. These results suggest that force-related information is retained in motor cortex in individuals with tetraplegia, and that it is feasible to incorporate cortical activity during attempted force production into iBCIs that restore hand grasping function.

86. FEP-PDMS Hybrid Microfluidic Devices for Light-Sheet Microscopy

Stephanie Reynolds, Thomas Levario, Daniel Porto, Yongmin Cho et al
Georgia Institute of Technology

Purpose Live model organisms such as *Caenorhabditis elegans* (*C. elegans*) are often handled and imaged in a high-throughput manner using microfluidic devices. Live imaging methods can include epifluorescence and confocal microscopy, but these can exhibit significant photobleaching and phototoxicity. Light-sheet microscopy (LSM) has recently been developed to limit the effects of photobleaching and phototoxicity. LSM utilizes a water-immersion lens in order to create a refractive-index-matched imaging axis for biological specimens ($n_{\text{water}} = 1.333$). However, microfluidic devices cannot be used with LSM, because typical devices are constructed with optically-incompatible materials including glass coverslips ($n_{\text{glass}} = 1.47$). Furthermore, the space between the two LSM objectives is rather small, preventing the use of large glass coverslips within the chamber. Thus, the purpose of this work is to develop microfluidic devices that are appropriately sized and optically compatible with LSM. Fluoroethylene propylene (FEP) sheets satisfy the refractive index requirements ($n_{\text{FEP}} = 1.344$), and can be easily cut to size. However, standard FEP has a very low surface energy and bonding with poly(dimethylsiloxane) (PDMS) is weak, but corona-treated FEP (Type C or Type C20) has some adhesive properties, making it ideal for this application. Methods Corona-treated FEP (Type C) is treated with APTMS in DI water, rinsed with DI water, and dried with air. Crosslinked PDMS is plasma treated for 1 minute and then placed on top of the corona-treated side of the FEP. Device was placed on a hot plate and temperature was ramped from RT to 260°C. A slight pressure was applied to secure the bond of FEP to PDMS. Results While our procedure still needs to be optimized, our preliminary results suggest that pressure-withstanding capabilities of the FEP-PDMS hybrid devices exceed 15 psi and are stable when submerged in water. Further proof-of-concept studies are still needed. Conclusion This FEP-PDMS device is a step toward integrating microfluidics with LSM for high-throughput imaging of *C. elegans* and other model organisms.

87. Chemotherapeutic Treatment Enriches for Cancer Stem Cell Content within Breast Cancer Spheroids

Daniel S. Reynolds, Kristie M. Tevis, Muhammad H. Zaman, Mark W. Grinstaff

Boston University

Purpose: Most in vitro tumor models fail to recapitulate the abnormal multicellular architecture of in vivo tumors or accurately predict in vivo cellular responses to therapeutics. Here, the purpose of this study was to assess the response of breast cancer cells to two front-line chemotherapies (paclitaxel and cisplatin) within an in vitro 3D collagen-embedded spheroid tumor model, which reflects the multicellular architecture of in vivo tumors, compared to a 3D collagen diffusely-embedded single-cell model and a 2D monolayer. Furthermore, because failure to completely eradicate the highly malignant cancer stem cell (CSC) subpopulation is thought to be a driver of cancer relapse, we also investigated the presence of CSCs across the three in vitro models. **Methods:** The triple-negative breast cancer cell line MDA-MD-231 was used in this study. For the 2D monolayer, cells were cultured on tissue-treated polystyrene. In the 3D diffusely-embedded model, cells were embedded within 4 mg/mL collagen gels at a seeding density of 105 cells/mL. For the embedded spheroid model, spheroids composed of 104 cells were formed following our published procedure and embedded in 4 mg/mL collagen gels. The treatment regimen for all models began 24 hours after initial seeding and consisted of 72 hours of drug exposure—10 ng/mL for paclitaxel (Indena) and 1.5 µg/mL for cisplatin (Sigma-Aldrich)—followed by removal of drug and an additional 72 hours of culture. Viability following drug treatment was assessed using a colorimetric MTS cell proliferation assay (Sigma) and oxoplates (Precision Sensing). CSC content was quantified using three methods: 1) ALDEFLUOR Stem Cell Identification Kit (Stem Cell Technologies); 2) Mammosphere Assay; and 3) RT-qPCR analysis of two CSC-related genes (ALDH1A3 and SOX2). **Results:** Viability measurements of the in vitro models revealed cells within the 2D monolayer condition to be the most sensitive to chemotherapeutic intervention, and cells remaining within the core of the spheroid, which we termed the ‘core’ population, to be the least sensitive. Moreover, the drug sensitivity of the 3D single-cell diffusely embedded model was similar to that of cells which had invaded away from the spheroid, termed the ‘periphery’ population. CSC quantification studies revealed CSC content to be inversely related to drug efficacy. The ALDEFLUOR assay, mammosphere assay, and RT-qPCR analysis of the untreated in vitro models showed the spheroid core population to have an enriched CSC subpopulation. Moreover, the high sensitivity of the TaqMan assay provided the means to assess ALDH1A3 and SOX2 expression across our in vitro models following treatment with either paclitaxel or cisplatin. Specifically, treatment with paclitaxel led to a statistically significant increase in both ALDH1A3 and SOX2 expression across all in vitro models compared to the untreated conditions; suggesting that paclitaxel treatment enriches for CSCs. Treatment with cisplatin significantly increased both ALDH1A3 and SOX2 expression within the 2D monolayer and 3D diffuse systems, but did not increase these genes within the core or periphery populations. **Conclusion:** In summary, we describe a tumor model that recapitulates the cell-cell and cell-matrix interactions found within the native tumor and its microenvironment. The results show that paclitaxel or cisplatin alone do not effectively combat

tumor growth—consistent with many clinical outcomes—and support the further evaluation of dual therapies involving cisplatin or paclitaxel in combination with ALDH inhibitors in order to target CSCs, which are linked to chemoresistance, recurrence, metastasis, and progression.

88. Multimodal-MRI based study of the effects of methylene blue in the human brain

Pavel Rodriguez, MD, Mary Woolsey, MS, Wilson B. Altmeyer, MD, Francisco Gonzalez-Lima, PhD et al
The University of Texas Health Science Center at San Antonio

Purpose: USP methylene blue USP (MB) is a FDA-grandfathered drug used in clinics to treat methemoglobinemia and as a surgical stain. Oral MB crosses the blood brain barrier and acts on the mitochondria to sustain or enhance ATP energy production. MB has shown efficacy in animal models of Parkinson's and Alzheimer's disease (AD). MB also increased oxygen consumption, brain glucose uptake, and fMRI evoked responses in the rat brain. A phase II clinical trial showed that daily oral doses of 300mg MB slowed the progression of AD compared to placebo using neurocognitive assessments. Our aim was to use task-based and task-free functional MRI (fMRI) to assess the efficacy of MB on cognitive and physiologic measures in the human brain. **Methods:** Study Design: Randomized, double-blinded, placebo-controlled clinical trial (Phase II) divided into two stages. **Primary Outcome Measures:** • Working memory fMRI task and behavioral measures • Episodic memory fMRI task and behavioral measures • Sustained attention task fMRI and behavioral measures • Visuomotor task cerebral blood flow (CBF) • Resting Functional Connectivity fMRI measures • Resting CO₂ Challenge **Secondary Outcome Measures:** • Neuropsychological Battery Stage 1 (NCT01836094) • Arms: MB (n=15) and Placebo Healthy Young (n=13), mean age 28-30 • Intervention: 280mg MB or placebo oral x 1 • Time Frame: 1 hour after completion of baseline fMRI tasks **Stage 2 (NCT02380573)** • Arms: MB Mild Cognitive Impairment (MCI) and Healthy elderly (40 total), Placebo MCI and Healthy Elderly (40 total), ages 65-89 • Intervention: MB (94 mg x 3 daily) or placebo oral with phenazopyridine (97.5 mg daily, all subjects) • Time Frame: baseline, 2 weeks ± 3 days, 12 weeks ± 7 days for all primary outcome measures. Follow up secondary outcome measures will be obtained at 2 weeks and 12 weeks **Statistical analysis:** Repeated measures analysis of variance (ANOVA) to assess Drug x Time between-group interactions **Results:** In healthy young subjects: MB significantly increased fMRI response in the bilateral insular cortex during the sustained attention task, and in the prefrontal, parietal and occipital cortex during the working memory task. MB treated subjects had a 7% increase in short-term memory retrieval (P=0.01). MB was also associated with a reduction in CBF in a task-related network during the VMT, and with stronger resting-state functional connectivity in multiple regions linking perception and memory functions. **Conclusions:** A low dose of MB can increase short-term memory retrieval, modulate sustained attention, working memory, resting-state and task-related visuomotor neural networks in the human brain. The findings support our ongoing investigations in elderly and MCI populations (Stage 2).

89. Lack of β -catenin in hepatocytes impairs proliferation and promotes liver stem cell-mediated repair in response to the choline-deficient ethionine-supplemented diet

Jacquelyn O. Russell, Hirohisa Okabe, Sucha Singh, Minakshi Poddar et al

University of Pittsburgh

Purpose: Despite the liver's capacity for regeneration, liver disease is the 12th leading cause of death in the United States. Treatments for chronic liver disease remain limited; thus, the purpose of this work is to elucidate mechanisms of liver regeneration. Typically, liver regeneration is mediated by proliferation of hepatocytes. When hepatocyte proliferation is impaired, liver stem cells (LSCs) are activated and are thought to mediate regeneration by differentiating into hepatocytes. However, the role and origin of LSCs remains controversial. The choline-deficient ethionine-supplemented (CDE) diet model of liver injury is known to induce proliferation of LSCs. However, since the CDE diet does not block hepatocyte proliferation, recent evidence has supported repair primarily driven by hepatocyte self-duplication in the CDE diet model. As a member of the WNT signaling pathway, β -catenin plays an important role in liver regeneration by promoting hepatocyte proliferation. Therefore, we hypothesize that β -catenin loss in hepatocytes would impair hepatocyte proliferation and lead to biliary-derived LSC-mediated hepatic repair in the CDE diet model. **Methods:** To determine the role of β -catenin in hepatocyte proliferation, we placed mice with genetic deletion of β -catenin in both biliary epithelial cells (BECs) and hepatocytes (Albumin-Cre β -catenin KO mice) on the CDE diet. To investigate whether liver regeneration is mediated by LSCs in this model, 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 β -catenin and permanently label hepatocytes with EYFP (AAV8 β -catenin KO mice). Importantly, in this model BECs contain β -catenin and do not express EYFP. **Results:** Albumin-Cre β -catenin KO mice display increased morbidity, mortality, and defective hepatocyte proliferation when compared to wild-type (WT) littermates after 2 weeks of CDE diet. Similarly, when AAV8 β -catenin KO mice were given two weeks of CDE diet they displayed increased liver injury and a lack of hepatocyte proliferation compared to β -catenin WT littermates. Notably, in AAV8 β -catenin KO mice given two weeks of CDE diet followed by a two week recovery on normal diet we detected clusters of hepatocytes which expressed β -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 β -catenin expression. **Conclusions:** Our results demonstrate that loss of β -catenin in hepatocytes impairs hepatocyte proliferation after CDE diet-induced liver injury and supports the hypothesis that LSCs mediate liver regeneration when hepatocyte proliferation is blocked.

90. Tissue-specific Effects of Inflammatory and Cancerous Esophageal Extracellular Matrix Hydrogels

Lindsey T. Saldin, Luai Huleihel, Madeline Cramer, Maria Quidgley-Martin et al

University of Pittsburgh

Purpose Despite the known importance of the microenvironment in cancer progression, cancer biology tools to recapitulate the microenvironment have largely remained unchanged: collagen gel and Matrigel have dominated the cancer biology literature for the past 50 and 33 years, respectively. The stimulus of the present study was to develop a new cancer biology technology, disease-specific ECM hydrogels. A novel approach was used to develop normal, inflammatory, and neoplastic ECM hydrogels from decellularized normal, inflammatory, and neoplastic adenocarcinoma (EAC) esophageal tissue and to identify mechanisms by which these ECM hydrogels influence cell behavior. We identified and isolated matrix-bound nanovesicles (MBVs) from ECM and showed their ability to rapidly and markedly affect cell phenotype. We will characterize MBVs derived from the three ECMs. Important and unanswered questions are: 1) What is the profile of extracellular miRNA, contained within the ECM via MBVs, to drive EAC progression? 2) How does diseased ECM activate an important cell type in an inflammatory driven cancer, the macrophages, via dynamic reciprocity? **Methods** Normal, inflammatory, and EAC tissue from a rat model of EAC were decellularized using the same protocol, assessed for absence of nuclei, and formed into hydrogels as previously described. SDS-PAGE/Silver Stain were used to characterize the protein profiles and scanning-electron microscopy was used to visualize the nanostructure of the three ECM hydrogels. The activation state (M1/M2) of human naïve macrophages exposed to the three ECM hydrogels in vitro was determined by gene expression (qPCR) and secreted products (ELISA). Small RNA sequencing was used to identify the miRNA profiles contained within normal, inflammatory, and neoplastic ECM MBVs. **Results** The three ECMs showed distinctive fiber networks and chromatographic protein profiles. Metaplastic and neoplastic ECM distinctively activate macrophages to a dual "pro-inflammatory" (TNFalpha high) and "immunomodulatory" (IL1RN high) state, with expression that increased as ECM tumorigenicity increased. MBVs were isolated from the three ECMs as a potential mechanism to regulate cell behavior. Top differentially regulated MBV miRNAs were notably related to epithelial-mesenchymal transition, a known mechanism of EAC progression, cancer, and macrophage activation suggesting the direct role of the ECM to reciprocally and dynamically instruct cell behavior. **Conclusion** A novel ECM hydrogel "progression series" was developed from normal, inflammatory, and neoplastic EAC tissue. A better understanding of diseased ECM MBV miRNA cargo profiles and disease-specific activation of macrophages will guide regenerative strategies for patients of this increasingly devastating form of cancer.

91. Perfluorocarbon doped hydrogels for tissue engineering applications

Daniela Y Santiesteban, Stanislav Emelianov, Laura Suggs

UT

Purpose: Stem cell (SC) angiogenic therapies seek to promote angiogenesis needed for restoration of injured ischemic tissues. Although initial SC studies showed promise, clinical translation has proven challenging due to high amounts of SC death upon implantation. The high amount of SC death can, in part, be attributed to the hypoxic implantation site and lack of host integration into scaffolds used for SC delivery. In order to produce clinically relevant tissues, maintaining survival of cells until blood vessel ingrowth occurs is essential. To achieve this, we have developed a nanoparticle-doped PEGylated fibrin gel that 1) allows for short-term increased oxygen (O₂) levels and 2) long-term control over hydrogel porosity which can impact stem cell differentiation, nutrient diffusion and improve host integration. **Methods:** All studies utilized human adipose stem cells (hASCs). A PEGylated fibrin hydrogel was used for the scaffold because it offers good SC viability and proliferation. Perfluorocarbon nanodroplets (PFCnDs) with a stabilizing shell were synthesized and incorporated into hydrogels. A 1064 absorbing dye (Epolig 3072) was encapsulated within the PFCnDs to allow for external triggering via lasing. To assess effects of lasing on cell viability, ASCs within gels were stained (LIVE/DEAD) 24 hours after lasing and imaged with a Zeiss LSM 710 confocal microscope. PFCnD's ability to deliver appropriate O₂ for maintaining ASC viability was assessed by culturing ASCs within doped hydrogels under hypoxic conditions (1% O₂) for 48 hours. **Results:** Conducted studies demonstrate the promise of a PFCnD-doped scaffold to enhance SC angiogenic therapies. PFCnDs allow for short-term oxygen delivery that allows for increased SC survival under hypoxic conditions (1% O₂). PFCnD doped hydrogels had significant higher viability than control groups at 72 hours. PFCnDs also allow for long-term control over porosity and mechanical properties of the hydrogel. Lasing of PFCnDs causes phase -changes of the particles, resulting in expansion which alters the hydrogel within close proximity and creates porous structures. Based on nD concentration and lasing energy, one could dictate the extent of porosity created. LIVE/DEAD staining was performed to ensure that lasing does not have any cytotoxic effects on cells. **Conclusions:** The combination of increased oxygen and dynamic hydrogel properties are expected to lead to more effective stem cell angiogenic therapies, as cells have increased viability and the changes in hydrogel properties can facilitate ingrowth of blood vessels and improve host integration.

92. Investigating the role of co-activators in inducible transcription at the single cell level

Andrew W. Sawyer, Michael T. Marr II

Brandeis University

The tight control of gene expression is achieved largely through the ordered assembly of large multiprotein complexes at the promoters of mRNA genes. For genes that respond to cellular stress signals, the amount of gene expression must be tightly coupled to the stress condition. We have been investigating the role of co-activators in the cellular response to heavy metals using the metallothionein genes as a model. Previous work from our lab showed that upon destabilization of TFIID, a core co-activator, we find an unexpected increase in transcription of these genes in response to heavy metal. These previous studies were done using methods that operate at population level and this can obscure the heterogeneity in the system. To investigate this we are using RNA FISH to examine the transcriptional dynamics following heavy metal treatment in the presence or absence of various co-activators. We find that the responding population is extremely heterogeneous, with huge variations in the number of RNAs per cell. In addition, the induction of transcription is not uniform, with only a fraction of the cells transcribing at any given time. We also find that upon destabilization of TFIID there is an increase in total RNA, similar to what was seen at the population level. However, with the single cell approach, we find that the regulation changes, not only in terms of number of RNAs per cell but also in the uniformity of the response across the population. There is a much tighter distribution in the number of RNAs per cell.

93. MRI evaluation of spinal cord lesions injected with a gelatin-based matrix in a rat model

Adhvait M Shah, Tehya Johnson, Myron Spector

Massachusetts Institute of Technology

Introduction: Spinal cord injury (SCI) is a devastating condition affecting roughly 253,000 patients in US. As a result of the injury, several functional impairments in breathing, bladder control and limb movements drastically reduce the patient's quality of life. Current treatments include administering steroids and rehabilitation. The root cause – traumatic loss or degeneration of neural tissue – is not targeted. Our overall goal is to assess the effectiveness of an injectable gelatin-based matrix with specific growth factors in spinal cord tissue repair. It is imperative to include in the preclinical animal investigation the non-invasive imaging modality that, in addition to be able to provide longitudinal assessments in the animal model, will also be able to be employed in an ultimate human trial. In this work employing a rat model, we present ex-vivo spinal cord MRIs with a proof of principle that critical characteristics of the injury site can be evaluated by MRI, and correlated with behavioral assessment of spinal cord injury. **Materials and Methods:** Twelve lewis rats (300 g) underwent survival surgery that induced a 1 mm T8 hemiresection injury to their spinal cord. On Day 0, the control group was injected with 15 μ l of gelatin-hydroxyphenyl propionic acid (Gtn-HPA) matrix (Gel only group, n=6) and the experimental group was injected with 15 μ l of Gtn-HPA matrix with epidermal growth factor (Gel + EGF group, dose = 6 μ g/rat). Behavioral data were employed from historical untreated controls. Every week until sacrifice, rats underwent open field locomotor test and their hindlimb function was rated as per the Beattie, Basso, Brashnahan (BBB) scale from 0 (complete paralysis) to 21 (normal gait). The rater was blinded to the groups. After 4 weeks, they were sacrificed by transcardial perfusion and relevant spine region was preserved in 4% PFA at 4°C. After sacrifice, T2-weighted coronal MRI images of the spinal cords with injury site were obtained in a 7T Bruker Scanner (resolution = 75 μ m, slice thickness = 250 μ m). Lesion volume was calculated using ImageJ. **Results & discussion:** We were able to obtain high-resolution MRI images showing the lesion site and the surrounding tissue in greater detail giving further insights (Figure 1). For Gtn-HPA + EGF group, average lesion volume was calculated to be 12.53 mm³, which was found to be lower than Gel only group, 15.27 mm³ (p = 0.05) (Figure 2). After 4 weeks, the Gel+EGF group rats showed greater functional improvement from 5.3 to 13.5 average BBB score (Δ = 8.2), while the Gel only group showed improvement from 5.3 to 11 (Δ = 5.7) (p = 0.05, Figure 3). Results from MRI correlate with the results from functional assessment of the rats using BBB scale. **Conclusion:** Our work provides a proof of principle that Gtn-HPA gel with EGF reduces the lesion volume contributing to greater protection of the surrounding healthy tissue. Moreover, our preliminary work suggests that MRI can be used as a tool to non-invasively study important critical parameters of the lesion site such as lesion volume. Encouraging results motivate us to further test various doses of EGF and relevant factors to induce tissue regeneration in our animal model. We aim to test a combinatorial approach with Gtn-HPA gel, EGF and a cell types such as bone-marrow derived mesenchymal stem cells.

94. Development of a Pipeline to Integrate High Angular Resolution Diffusion Imaging (HARDI) and Intracranial EEG Data in Epilepsy Patients

Preya Shah, Lohith Kini, Ankit Khambhati, Brian Litt et al

University of Pennsylvania

Purpose: Diffusion tractography is an MRI-based technique which can probe the brain's structural connectivity and identify white matter abnormalities in epilepsy patients. Intracranial EEG (iEEG) data, which is commonly recorded in patients with intractable epilepsy, can provide valuable information about the brain's functional connectivity. Integration of the two data types may allow us to better understand the relationship between structural and functional epileptic networks in the brain, and more robustly identify areas of seizure onset and spread. With this motivation, we have developed a pipeline to facilitate combined analysis of diffusion tractography and iEEG connectivity data. For our structural data, we utilize High Angular Resolution Diffusion Imaging (HARDI), an advanced diffusion imaging method which can produce reliable tractography results in regions of crossing white matter pathways. **Methods:** We have collected CT, T1 MRI, HARDI, and iEEG data for five patients with intractable epilepsy. A pipeline was designed using data from one patient implanted with 78 subdural electrodes. HARDI data was acquired on a Siemens Trio Magnetom 3T scanner using 116 diffusion weighted directions, 2.0x2.0x2.0mm voxel size, and b-values of 300 s/mm², 700 s/mm², and 1000 s/mm². Tractography was performed using q-space diffeomorphic reconstruction. Structural connectivity matrices were generated using the number of tracts connecting each region. Functional connectivity matrices were generated by calculating cross-correlations between iEEG electrode recordings. **Results:** Via thresholding and coregistration, we successfully localized and determined coordinates of the patient's 78 electrodes. By creating cylindrical regions around each electrode, we were able to perform HARDI tractography using electrodes as seed regions. The tractography reveals evidence of connectivity between the electrode regions and the brain's major white matter pathways. Moreover, we generated a functional adjacency plot using ictal iEEG connectivity data. In this framework, both structural and functional connectivity are characterized using electrodes as nodes. Therefore, these data can be used as the basis for future direct quantitative comparison of HARDI and iEEG data. **Conclusion:** We have outlined a process to enable multimodal analysis of brain connectivity in epilepsy. To our knowledge, this is the first attempt to directly integrate HARDI and iEEG data. We hope to use the process to further analyze epileptic networks in our growing repository of patient data. We believe that a combined structural-functional approach can be extremely valuable in better localizing seizure onset, predicting pathways of seizure spread, and ultimately informing clinical decision making.

95. Invadosome formation and function in chemotrophic axon guidance

Caitlin A. Short, Edwin A. Suarez-Zayas, Timothy M. Gomez,
University of Wisconsin, Madison

Invadosomes are F-actin rich adhesions which protrude the membranes of some migrating cells and promote degradation of the extracellular matrix. Although invadosomes have predominantly been studied in cancer and immune cells they have recently been identified in neural crest cells in the developing zebrafish and pathfinding growth cones of *Xenopus laevis* spinal neurons (Santiago et al., Development). Our lab found that similar to non neuronal cells, invadosome formation in neuronal growth cones requires Tks5 and that active Src (pY418-Src) localizes to these sites. Our previous work largely examined invadosomes in 2D culture, however, in vivo growth cones may receive cues from a variety of directions in 3D to modulate their growth and form networks. In this work we employ confocal and structured illumination microscopy (SIM) of developing motoneurons (MN) in both 2D and 3D extracellular protein environments to test the roles of candidate morphogens and investigate the formation and function of growth cone invadosomes in neuron growth and axon guidance. We hypothesize that growth factors, such as BDNF and SDF-1, are released from peripheral tissues during development to induce invadosome formation in MNs to guide them through the basement membrane of the spinal cord and into the periphery. Preliminary data suggests that axons are guided along a gradient of BDNF in collagen gels. Invadosome-dependent penetration of collagen is being examined by manipulating the activity of Tks5, Src kinase, and the actin regulatory Rho GTPase cdc42. Future work will continue to elucidate the molecular induction of growth cone invadosomes.

96. Segmentation of dense cellular microscopy images for quantification of inflammation in lupus nephritis

Adam Sibley, Maryellen Giger, Yulei Jiang

University of Chicago

Purpose To develop a computerized segmentation method for densely packed cells in microscopy images. This enables analysis of the process of B cell activation by T follicular helper cells in lupus nephritis and in inflamed human tissue in general. We primarily desire accurate localization of cells and accurate representation of their boundaries to enable shape and distance analysis of interacting cells.

Methods and materials T follicular helper cells are critical for B cell activation in germinal centers and are often observed in human inflamed tissue. However, it is not known whether they contribute to in-situ inflammation. Using confocal laser scanning microscopy with immunofluorescent antibody staining, we have previously developed a method to quantify image cell interaction by segmenting image channels corresponding to the different cell types and using shape and distance metrics. We seek to validate this model in a mouse model of tonsil germinal centers. On 42 cellular images of interacting cells in a mouse model, we used a quincunx wavelet transform with gaussian filtering applied to the wavelet coefficients at each scale. The coefficients at each scale are then multiplied together to produce a wavelet multiscale product. Thresholding this product produces a robust segmentation of key features in the cell. We then apply an iterative morphological opening with a circular structuring element and as processing proceeds, a selection criterion based on solidity. Objects above a solidity threshold are removed from consideration at each iteration. In order to differentiate the objects, mean shift clustering is performed on the centroid features of connected object populations. This reliably identifies object clusters corresponding to actual cells without requiring foreknowledge of the number of cells present. Final object segmentations are a combination of segmented objects in the differentiated clusters. Intra-cluster outliers are removed based on centroid, area, solidity, and boundary saliency features. Results Our segmentation results compare favorably against our previous method using the watershed transform. By visual inspection this method produced poor cell localization and poor cell shape characterization.

Conclusions With increased accuracy of our cellular segmentation method for dense tissue we can better quantify cell position and shape in our images. This method will aid in segmentation of cells in dense tissue in human renal biopsies to quantify inflammation in lupus nephritis.

97. Discovering Temporal Signatures that Predict Disease Trajectory of Glioblastoma Multiforme Patients

Nova F. Smedley, Dr. William Hsu, Dr. Timothy F. Cloughsey, Dr. Benjamin M. Ellingson

University of California, Los Angeles

Purpose Patients with glioblastoma multiforme (GBM) experience poor prognosis. Surgery and adjuvant therapies following diagnosis improves median survival by 6 months. Unfortunately, tumor recurrence is essentially inevitable and subsequent rounds of therapies have mostly proven ineffective. The decision to treat or not treat is informed by a patient's clinical history and disease trajectory. While a variety of clinical and imaging observations (e.g., Karnofsky performance score (KPS), tumor volume) are reviewed by a neuro-oncologist, the relationship between temporal patterns captured in these observations and overall survival is not well understood. Our goal is to utilize sequential pattern mining approaches to identify temporal signatures that are predictive of irreversible patient decline after observing the pattern.

Methods Between September 1999 to August 2014, a UCLA cohort of 314 newly diagnosed GBM patients were followed from time of diagnosis until May 11, 2015. During this period, tumor volume from radiological images, surgery, chemotherapy, radiation, and several forms of neurological evaluations were collected. The dataset has a total of 9,297 clinical visits and 23,963 events. Representing each patient as a sequence of events, frequent sequences were mined using the cSPADE algorithm. Several forms of constraints, including temporal gaps between events, and data discretization were utilized to identify meaningful frequent sequences, or patterns. Patterns were then analyzed through the Kaplan-Meier (KM) estimator to identify changes in survival days from when the pattern was observed. Significant differences in survival was controlled by the false-discovery rate method. Cox proportional hazard regression using covariates from patient demographics and genomics (e.g., MGMT methylation, IDH1 mutation) identified the predictive value of each sequence. Results cSPADE found 309 patterns consisting of 598 single events, 183 two-event sequences, and 18 three-event sequences. A total of 87 patterns were identified to be significant predictors of survival. Examples of these patterns include a) an overall neurological score of -1 on the same day as a decrease in KPS from the last clinical visit, b) a tumor volume increase followed by a tumor volume increase within the next month, and c) a decrease in KPS, followed by the same KPS within the next month.

Conclusions This work presented a set of sequential patterns extracted from a relatively large cohort of GBM patients throughout their history of clinical observations. These patterns were able to predict patient decline in addition to being intuitive and understandable.

98. Fast Sequence Search using SBT

Brad Solomon, Carl Kingsford

Carnegie Mellon

Purpose An enormous amount of DNA and RNA short read sequence data has been published worldwide. By aggregating and analyzing this data as a whole, it would be possible to investigate genetic variation, and condition- and disease-specific gene function in ways the original depositors of the data did not anticipate. Unfortunately the scale of the data is so large that it is not even possible to search for a single query sequence in reasonable computational time. **Methods** We developed a novel data structure, the Sequence Bloom Tree, to address this gap between data and analysis. We demonstrate SBT's efficiency by building an index on 2652 human RNA-seq experiments and searching this index for all 214,293 known human transcript in under four days using only megabytes of RAM and a single CPU. We also benchmark SBT's average query time and index size to several existing tools such as Sailfish, STAR, and SRA-Blast. **Results** We find that an average single transcript search takes SBT 20 minutes using 239 MB of RAM and a single thread. The comparable search times using SRA-BLAST or STAR is 2.2 days or 921 days respectively, although both tools return alignments while SBT does not. SBT and STAR can also be batch queried, with SBT roughly 4,056 times faster than STAR. SBT achieves this speedup while using 4% of the original data storage cost in a directly searchable index. These significant speed and size benefits come at a minor accuracy loss, with an average true positive rate of up to 0.85 when using Sailfish gene expression estimates as a ground truth. **Conclusion** Currently, it is difficult to access all the relevant data relating to a particular research question from available sequencing experiments. SBTs enable the efficient mining of these data and could be used to uncover biological insights that can be revealed only through the analysis of multiple data sets from different sources. Furthermore, SBTs do not require prior knowledge about sequences of interest, making it possible to identify, for example, the expression of unknown isoforms or long noncoding RNAs. This algorithm makes it practical to search large sequencing repositories and may open up new uses for these rich collections of data. This work was reported in Solomon, Brad, and Carl Kingsford. "Fast search of thousands of short-read sequencing experiments." *Nature biotechnology* (2016).

99. Design and Prototype Development of a Torsional Ventricular Assist Device (tVAD)

Elaine Soohoo, Lewis K. Waldman, Dennis R. Trumble

Carnegie Mellon University, Department of Biomedical Engineering

Purpose Our goal is to develop a torsion-based ventricular assist device (tVAD) as an alternative to traditional ventricular assist devices (VADs) currently available on the market. The tVAD attaches to the apex of the heart and will rotate in synchrony with the heartbeat with the intention of reducing wall stress and increasing the ventricles' ability to empty more completely. **Methods** The tVAD prototype was designed using a commercial CAD software package (Solidworks 2015, Dassault Systèmes Solidworks Corp., Waltham MA). The overall design approach was guided by computational simulations of applied apical torsion of the heart and results from in vivo pig experiments wherein a first generation tVAD rotated a hypokinetic heart a quarter turn during the systolic phase of the cardiac cycle. Parametric computational simulations were performed using ContinuityPro software (Insilicomed, Inc., La Jolla, CA) and used to determine design parameters for a second-generation tVAD prototype. These simulations utilized beating heart models attached to a closed-loop circulatory system. Ventricular size, shape and dimensions were based on anatomic measurements taken from both adult porcine hearts and human heart failure patients, while biomechanic and circulatory model parameters were taken from literature values. **Results** We have recently created a working second-generation tVAD prototype design and surgical delivery scheme suitable for clinical use. Model details will be fine-tuned prior to device manufacturing based on performance parameters derived from further computational simulations. Initial results from a parametric study looking at the effects of increasing rotation on the ventricles demonstrated that when compared to a clinical heart failure model, with increasing applied apical rotation (up to 75°), there is an increase in both the ejection fraction (35.2%) and stroke work (47.2%). **Conclusion** Based on results from preliminary computational simulations and experiments on live porcine hearts, applied apical torsion shows promise as an alternative method to traditional cardiac assist devices currently used to treat congestive heart failure.

100. Pointwise Mutual Information Quantifies Intra-Tumor Heterogeneity in Tissue Sections Labeled with Multiple Fluorescent Biomarkers

D.M. Spagnolo, R. Gyanchandani, Y. Al-Kofahi, A.M. Stern et al

University of Pittsburgh

Spatial intra-tumor heterogeneity measures are potentially important diagnostic biomarkers for cancer progression, proliferation, and response to therapy. Spatial relationships among cells including cancer and stromal cells in the tumor microenvironment (TME) are key contributors to heterogeneity. We demonstrate how to quantify spatial heterogeneity from immunofluorescence pathology samples, using a set of breast cancer biomarkers. We learn a set of dominant biomarker intensity patterns and map the spatial distribution of the biomarker patterns with a network. We then describe the pairwise association statistics for each pattern within the network using pointwise mutual information (PMI) and visually represent heterogeneity with a two-dimensional map. PMI is generalizable to highly multiplexed immunofluorescence images, as well as spatial data from complementary in situ methods including FISSEQ and CyTOF, sampling many different components within the TME [1, 2]. We hypothesize that PMI will uncover key spatial interactions in the TME that contribute to disease proliferation and progression. 1. Lee, J.H., et al., Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues. *Nat Protoc*, 2015. 10(3): p. 442-58. 2. Giesen, C., et al., Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods*, 2014. 11(4): p. 417-22.

101. Imaging approaches to detect & monitor changes in joint architecture & brain networks in TMJ pain

Megan M. Sperry, Sonia Kartha, Ya-Hsin Yu, Eric J. Granquist et al

University of Pennsylvania

PURPOSE. Temporomandibular joint (TMJ) osteoarthritis is a common, low-grade inflammatory condition that has a multifactorial etiology including pain. In most patients, pain resolves without needing further clinical action. However, for a subset of patients, chronic TMJ disorder develops, with long-lasting symptoms. Sustained neuronal hyperexcitability throughout the central nervous system is a major contributor to the development of chronic pain and can lead to changes in brain circuitry. This study investigates alterations in the TMJ and brain using quantitative in vivo imaging techniques to compare different pain states. **METHODS.** All procedures were IACUC-approved. Repeated mouth-opening was imposed daily for one week in female Holtzman rats under isoflurane anesthesia to induce either resolving or persistent pain (n=7/group). Orofacial pain was assessed by measuring mechanical hyperalgesia. CT images of the TMJ were acquired before loading (baseline) and at day 14, to quantify joint structure. Image stacks were registered between days, automatically segmented, and reconstructed in 3D. Changes in joint architecture were evaluated using image subtraction between registered images. In addition, FDG-PET images of the brain were acquired at baseline and day 7 to evaluate metabolic activity when the pain responses diverge. Images were registered to Schwarz's rat brain template. Networks were created by representing each brain region as a node; edges connecting nodes were defined by the Pearson correlation coefficient between FDG uptake in two regions for all rats. Meso-scale structure of the network was evaluated by the Louvain algorithm. **RESULTS.** Orofacial sensitivity is established during the loading period for both groups ($p < 0.001$), but only remains at day 14 in the persistent pain group ($p < 0.001$). Flattening of the TMJ condylar head is evident at day 14 only in the persistent painful group; this change in bone shape is not evident in the other group. Image subtraction reveals image intensity changes between day 14 and baseline that are larger in the painful group (1785 ± 621) compared to the group (1232 ± 542) with no pain at that time. In combining both groups, the modularity of the brain networks significantly decreases ($p < 0.0001$) at day 7 when pain is present. However, a change in community structure emerges in the sustained pain group at day 7 that is not evident in the resolving pain group. **CONCLUSIONS.** Both peripheral tissue and brain are altered when TMJ pain persists. These findings suggest that these, and/or other imaging approaches, may have predictive value in diagnosing disease (i.e. pain) progression in TMJ osteoarthritis.

102. Kupffer Cell Subsets Differ Between Young and Aged Murine Livers

Elizabeth C. Stahl, Bryan N. Brown

University of Pittsburgh

Purpose: The immune system, and in particular macrophages, are implicated in wound healing, pathogen clearance, and cancer progression. Studies show that tissue-resident macrophages become dysfunctional with aging, likely contributing to mortality in the elderly population. The liver contains approximately 80% of the total tissue-resident macrophages, known as Kupffer cells. Kupffer cells can be divided into two F4/80+ macrophage subsets: embryonic derived CD68+ and bone marrow derived CD11b+ cells, with different phenotypes and functions. Currently, it is unclear how these macrophage subsets are affected by aging and the implications for human health. We expect that advanced age will promote the accumulation of CD11b+ “bone marrow derived” Kupffer cells in the liver that exhibit cellular dysfunction, which may have implications for the overall health of aged hosts. **Methods:** We characterized differences in Kupffer cells from young (2-4 month) and aged (18-24 month) C57/Bl6 wild-type mice using immunofluorescent histological staining as well as flow cytometry for the macrophage markers F4/80, CD11b, CD32, and CD68. In addition, we examined macrophage function by polarizing cells to classically or alternatively activated phenotypes (M1 & M2) and measuring phagocytosis of e.coli particles in vitro. **Results:** Histological analyses showed the number of F4/80+ and CD68+ cells significantly increased with aging, demonstrating an overall increase in the number of macrophages residing in the liver. In addition, the area of CD32+ staining, which marks both macrophage progenitor and endothelial cells, remained consistent with age. Flow cytometry analysis confirmed differences in the macrophage subsets between young and aged murine livers, with a significant increase in CD11b+ macrophages, suggesting an increase in bone marrow origin of the tissue resident macrophages. Finally, functional analyses showed that aged Kupffer cells did not significantly polarize to an M1 or M2 phenotype, but were more phagocytically active than young Kupffer cells at baseline. **Conclusions:** The overall increase in macrophages in the aged liver and increased phagocytic activity suggests Kupffer cells may be affected by inflammatory systemic cues characteristic of aging and this may disrupt normal liver homeostasis. The significant increase in CD11b+ macrophages in the liver supports the hypothesized progressive replacement of embryonic macrophages with bone marrow derived macrophages in advanced age. Further characterizing the functional and phenotypical differences in the subsets of Kupffer cells during aging will increase our understanding of the host response to infection and cancer and aid in better design of therapies that will extend the healthspan of the population.

103. Optimizing unanesthetized cerebral oxygen consumption measures: comparison of MRI and near-infrared spectroscopy (NIRS) approaches in neonates with congenital heart disease

Jeffrey N Stout, Silvina L. Ferradal, Lilla Zollei, Divya S Bolar et al
Massachusetts Institute of Technology, HST

Purpose: Moderate to severe congenital heart disease (CHD) affects 6/1000 live births, with severe CHD resulting in adverse neurodevelopmental outcomes in over 50%. The etiology of neurodevelopmental disorders is unknown but evaluation of the hemodynamic state of CHD infants pre- and post-surgically has become a focus with the cerebral metabolic rate of oxygen consumption (CMRO₂) identified as a key parameter for clinical evaluation. We present MRI and NIRS measures of cerebral hemodynamics in nine stable neonates with CHD. MRI measures were performed without anesthesia and NIRS measures were performed at the bedside within one day of MRI without anesthesia. MRI and NIRS measures are compared to literature values. Methods: MRI and NIRS studies were performed at Boston Children's Hospital with IRB approval and parental consent. Both studies (N=9, age=4.8±2.5 days, 8 male, 1 female) took place in the pre-operative period within 12 hours of each other. The MRI protocol: Structural imaging (volume navigated MPRAGE and time of flight angiogram) was used to position velocity encoded phase contrast imaging to permit cerebral blood flow (CBF) calculation (TE/TR=4.67/16.65ms, resolution=0.5x0.5x4.0mm, velocity encoding=100cm/s, Tacq=1:19) and T2-relaxation under spin tagging (TRUST) measurements of oxygen saturation in the superior sagittal sinus (TE/TR=15/5000ms, resolution=2.3x2.3x5mm, inversion time=1025ms, tagging width=50mm, tagging gap=15mm, Tacq=1:19). The NIRS protocol: Frequency-domain near-infrared spectroscopy (FD-NIRS) and diffuse correlation spectroscopy (DCS). FD-NIRS provided regional measurements of oxygenated and deoxygenated hemoglobin, used to compute cerebral oxygen saturation. DCS provided a measure of microvascular blood flow. Results: MRI results are reported as mean ± standard error, and NIRS results as median (interquartile range) to permit comparison with previous literature: Venous oxygen saturation (SvO₂) was 55.4 ± 10.5% and 57.6 (51-59.9)%. CBF was 13.6 ± 2.3 ml/100g/min and 2.05 (1.53-2.28) cm³/s. CMRO₂ was 39.6 ± 9.5 μmol O₂/100g/min and 22.2 (19.2-24.2) ml O₂*cm³/dl/s. Discussion: Compared to other studies (P. Liu, et al., NMR in Bio, 2014. V. Jain and E. M. Buckley, JCBFM, 2014. M. Dehaes, et al., Bio Opt Exp, 2015.) our results show expected decreased mean CMRO₂ compared to healthy neonates and slightly higher CMRO₂ than in anesthetized subjects. SvO₂ measurements correlate across modalities (P = 0.003, R² = 0.74) suggesting that SvO₂ measures capture similar information and/or are relatively stable in this cohort. However, there was no significant correlation between CBF and CMRO₂. Conclusion: MRI and NIRS provide complementary methods for quantification of cerebral hemodynamics that if cross-validated would increase our confidence in both

modalities and lead to more comprehensive clinical monitoring. However, before data between these two modalities can be compared or combined, additional studies are needed to better understand the relationship between large vessel bulk flow and microvascular red blood cell flow. SvO₂ measurements in our study are significantly correlated between modalities, but CBF, and therefore CMRO₂, are not in good agreement either due to differences in physiology or biases in these measurements.

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104. A Parallel Approach to Energy Minimization of Protein-Ligand Interaction

Jocelyn Sunseri, David Ryan Koes

Carnegie Mellon University - University of Pittsburgh Computational Biology

Fundamental physical limitations associated with processor design have reduced the single-threaded efficiency gains in software performance that were previously achieved by innovations in hardware. This has led to increased reliance on parallel execution as a means of accelerating program performance, catalyzing the rapid development of hardware capable of executing single program, multiple data (SPMD) instruction streams efficiently. In particular, graphics processing units (GPUs), previously developed primarily for graphics rendering, have found diverse applications in scientific computing. Knowledge of underlying hardware and modifications of sequential algorithms to make them amenable to parallelization are required to yield significant gains from this approach. We report our algorithm for performing energy minimization of a protein-ligand molecule pair on a GPU, as well as relevant details of its implementation. We report a XX speedup over a CPU implementation of our algorithm. This type of energy minimization is useful for protein-ligand pose prediction (including docking) as well as binding free energy estimation, which is applicable to scoring putative hits in a virtual screen. Our implementation increases molecule screening throughput and is of particular value in performing real-time minimization requests made via our webserver, pharmit.

105. An injectable block copolymer synthetic cartilage

Stefanie A. Sydlik, Meng Deng, Cato T. Laurencin, Robert S. Langer

Carnegie Mellon University

Purpose. Synthetic materials hold the promise of biocompatibility, reduced foreign body response, and elimination of compliance mismatch in the field of tissue regeneration. Despite these promises, major limitations still exist in the materials used today, especially in cartilage repair. In this proposal, we present a new type of synthetic cartilage that mimics the chemical structure, namely type-II collagen and glycosaminoglycans, and ordered morphology of cartilage to reproduce the extraordinary material properties. **Methods.** A collagen mimetic peptide (CMP), based on repeating trimmers of GOP and capped with K residues was developed. This peptide self-will be verified by circular dichroism (CD). Further, the free amines are used to crosslink with aldehydes in modified hyaluronic acid (HA) or NHS-esters in chondroitin sulfate (CS). Maintenance of this collagen-mimetic morphology when crosslinked with HA or CS in the hydrogel was confirmed by SEM, and mechanical properties of the material were monitored over time using rheology. Human mesenchymal stem cells (hMSCs) were cultured on or crosslinked within the gel to study their differentiation. **Results.** CD and SEM confirms the maintenance of the collagen-like triple helix conformation of the peptide. SEM shows nanofibular morphology in the crosslinked gel, with fibers on the order of nanometers, self assembling into larger, micron sized domains of the copolymer. Rheological study showed that the mechanical properties of the gel can be tuned to 50- 200 kPa, which is on the order of native cartilage. Enzymatic degradation was found with collagenase, chondroitinase, and hyaluronidase. Human mesenchymal stem cells (hMSCs) were cultured on or crosslinked within the gel and show good morphology and suggest chondrogenic differentiation. An ex vivo implant model showed good integration and adherence to native cartilage. **Conclusion.** A thermoset block copolymer has been developed that mimics the major chemical motifs in cartilage, namely, type II collagen and glycosaminoglycans through the use of a collagen mimetic peptide (CMP) and hyaluronic acid (HA) or chondroitin sulfate (CS). Self-assembly of nanofibers and nanophase separation of the blocks give a predictable morphology, mimicking the ordered morphology found in native cartilage. The shear mechanical properties of the hydrogel approach can be tuned to match those of cartilage, and remain stable through in vitro experiments as cells break down the material at the same rate new extracellular matrix is built. While we have achieved very promising in vitro results, we have yet to prove this system in vivo.

106. A semiautomatic noninvasive technique for quantitative assessment of collateral circulation

Elizabeth Tong MD, Max Wintermark MD

University of California San Francisco

Purpose Collateral circulation plays a pivotal role in the pathophysiology of acute ischemic stroke, treatment outcome and clinical outcome. Collateral status is increasingly recognized as a promising biomarker for predicting the outcome of stroke. However, there is no single established grading system. The goal of our study is to propose a noninvasive technique for quantitative assessment of collateral circulation and investigate the prognostic value of our proposed collateral-score. **Methods** An original collateral-assessment software based on Perfusion CT was developed to semi-automatically (1) map out the collateral vessels, (2) determine the vascular origin of the collaterals, and (3) calculate the contribution from each vascular territory. A collateral score reflecting the contribution from each vascular territory (MCA, ACA and PCA) is computed. Patients from a stroke registry with anterior-circulation occlusion were retrospectively identified and their collateral scores were assessed. The correlation between collateral score with clinical outcome (as measured by 90-day modified Rankin Scale) and radiologic outcome (as measured by volume of infarct core) was investigated. **Results** A total of 100 patients, mean age 68 years, with M1 and/or M2 occlusion were included. Mean collateral score is significantly different between the stroke hemisphere and the normal (contralateral) hemisphere. The variance in the collateral score increases with age. Good collateral score is associated with smaller infarct core volume and lower 90-day modified Rankin Scale. Poor collateral score is associated with larger infarct core volume and higher 90-day modified Rankin Scale. **Conclusion** Good collateral status is associated with lower modified Rankin Scale at 90-day and smaller final infarct volume. Therefore evaluation of collaterals is important in stroke management. Our new collateral-assessment software allows non-invasive, objective, and quantitative assessment of collaterals.

107. Transurethral MR-guided high-intensity ultrasound system for focal ablation of prostate cancer

Trivedi, Hari, Partanen, Ari, Wood, Bradford, Choyke, Peter et al

UCSF

MR-guided transurethral ablation of prostatic tissue may be more accurate and safe than transrectal approaches. The objective of this study was to develop and validate a transurethral ultrasound system for focal ablation of regions within the prostate while limiting damage to urethral or peri-prostatic tissue. The MR-guided ultrasound system consists of an axially rotating applicator under robotic control with eight 0.5cm elements ($f=3\text{MHz}$, max. $P_{ac}=4\text{W}$). Each element can be modulated as the applicator is rotated to contour the ablation zone. Degassed water is circulated to cool the applicator and adjacent urethral tissue. Ablation zone is controlled through real-time feedback using PRF-based temperature monitoring. Preliminary testing using all eight elements at 50% power (0, 60, and 90 degree sweeps) was conducted in tissue-mimicking phantom. Ablation zones (defined as 240CEM43) were achieved to a depth of 4cm, while sparing a 0.5cm zone around the applicator. Sweeps resulted in ablation zones 5-10 degrees larger on either side than the programmed arc. An ablation zone of $0.8 \times 1.0\text{cm}$ was achieved in 60 seconds without rotation. Transurethral MR-guided high-intensity ultrasound therapy can accurately ablate targeted volumes while limiting thermal exposure in the near and far field. Pre-clinical trials have been completed and clinical trials are underway.

108. Extracellular Matrix Hydrogel Promotes Tissue Remodeling, Arteriogenesis, and Perfusion in a Rat Hindlimb Ischemia Model

Jessica L Ungerleider, Todd D Johnson, Melissa J Hernandez, Dean I Elhag et al

University of California, San Diego

Purpose: The prevalence of peripheral artery disease (PAD) is increasing and can lead to critical limb ischemia (CLI), ultimately increasing the risk of potential limb amputation. Currently, there are no therapies for PAD to effectively treat all of the underlying pathologies, including reduced tissue perfusion and muscle atrophy. This study aimed to examine acellular extracellular matrix based hydrogels as potential therapies for treating PAD. We tested the efficacy of using a tissue-specific injectable hydrogel, derived from decellularized porcine skeletal muscle (SKM), compared to a new human umbilical cord derived matrix (hUC) hydrogel. The latter could have greater potential for tissue regeneration due to its young tissue source age. **Methods:** In a rodent hindlimb ischemia model, both hydrogels were injected 1-week post-surgery, and perfusion was regularly monitored with laser speckle contrast analysis (LASCA) for 35 days post-injection. Immunohistochemistry and histology were used to assess neovascularization and muscle remodeling. **Results:** Quantitative proteomic analysis showed that both SKM and hUC contained complex, tissue-specific compositions. Significant improvements in hindlimb tissue perfusion and perfusion kinetics were observed with both biomaterials. End point histology indicated this was a result of arteriogenesis, rather than angiogenesis, and verified the materials were biocompatible. Furthermore, muscle fiber analysis showed the tissue specific matrix (SKM)-injected animals had muscle fiber area and circularity most closely resembling healthy contralateral muscle. **Conclusion:** These results show the efficacy of an injectable ECM hydrogel alone as a potential therapy for treating patients with PAD. They also suggest that non-tissue specific responses such as vascularization can be stimulated with a non-tissue specific ECM hydrogel, but a tissue specific ECM hydrogel may better influence muscle regeneration and remodeling.

109. Phantom feasibility study for utilization of crawling wave elastography to improve diagnosis of neonatal intracranial hemorrhage

Alexander M. Vezeridis, Kenneth Hoyt, Clark Z. Wu, Robert F. Mattrey

UC San Diego

Purpose: Neonatal cranial ultrasound (US) is an imaging test commonly performed to diagnose intracranial hemorrhage (ICH) in newborns. While an excellent test for detection of severe ICH, cranial US has very poor sensitivity and interobserver agreement for grade I and grade II ICH. The aim of our work is to improve detection neonatal ICH through the use of ultrasound elastography, specifically crawling wave elastography, by measuring stiffness of clot compared to surrounding brain parenchyma and cerebrospinal fluid (CSF). **Methods:** Agarose phantoms were crafted to hold clotting blood. Citrate-anticoagulated rabbit blood was introduced into the phantom, and a blood clot was formed by the addition of calcium chloride solution and thrombin enzyme. Before and the process of clotting, crawling wave elastography was employed to dynamically assess the shear modulus of the blood or forming clot at time points 5, 10, 20, 30, 50, 90, 120 minutes. Imaging of the crawling wave was performed using two different methods: (1) a Philips iU22 ultrasound machine using power Doppler magnitude estimation, and (2) a Verasonics ultrasound system using power Doppler variance estimation, which was hypothesized to be more sensitive to the low shear wave velocities anticipated in blood clots according to prior studies. **Results:** Using power Doppler magnitude estimation, crawling waves were not observed within clotting blood. Using power Doppler variance estimation, low crawling wave signal was observed in the clotting blood within 5 minutes of starting clotting, but not in unclotted blood. By the variance estimation method, crawling wave signal was observed to increase over time and plateau at maximum levels at 50 minutes after beginning clotting. **Conclusions:** Crawling wave elastography with variance estimation methods demonstrates the sensitivity required to assess blood clot stiffness during clotting, as evidenced by increased crawling wave signal over time during clotting. With further study, such methods may prove useful in distinguishing clotted blood from CSF (which does not propagate crawling waves) and normal brain parenchyma.

110. Using online variant calling for more accurate read mapping

Tim Wall, Carl Kingsford,
Carnegie Mellon University

The massive influx of next-generation sequencing (NGS) data over the past several years has given rise to two key problems in the field of NGS analysis: read mapping, or determining where on a template transcript a read could have originated; and variant calling, in which read alignments are used to determine potential genomic differences between a template transcript and the transcript from which the short reads are sequenced. Numerous approaches have been developed for both concepts; read mappers like Bowtie and BWA, among others, provide fast and accurate read alignments, while variant calling tools from SAMtools and GATK are effective at accurately calling variants. However, current read mappers are prone to template bias, as deviations from the template are 'edited' in the process of mapping, so true variants are not taken into account when editing the read, which can cause a deviation from true mappings. We propose a new mapper, NIRMAL, which combines a "lightweight" mapping algorithm augmented with a skip list data structure that allows potential variants to be efficiently stored and queried during mappings. By tracking potential variants during the mapping process, "true" variants can be determined and queried online, allowing for potentially more accurate mappings. This method could potentially prove highly useful in applications such as cancer genome variant detection and determining potential variants among closely related species.

111. On-Axis Acoustic-Radiation-Force-based Quantitative Stiffness Estimation in Phantoms

Kristy Walsh, Mark Palmeri, Brett Byram

Vanderbilt University

Purpose: In shear wave elasticity imaging (SWEI), stiffness can be estimated by measuring shear wave velocity at locations away from the acoustic radiation force (ARF) axis [1]. Instead, this research estimates stiffness by measuring the time-to-peak displacement directly along the ARF axis, which reduces hardware and sequencing complexity. We have shown previously in simulation results that an advanced displacement estimator reduces variability in the final stiffness estimate [2]. Here, we test the on-axis approach in 15 phantoms. **Methods:** We assume the phantoms are homogeneous, isotropic, and linearly elastic, thus time-to-peak displacement is directly proportional to shear wave speed. Since shear wave speed is directly related to shear stiffness, we create a stiffness look-up table of the time-to-peak displacement as a function of depth. We generated look-up tables using a 3D FEM model coupled to Field II simulations and the selected displacement estimation method. We simulated time-to-peak look-up tables for shear moduli from 1-15 kPa and attenuation of 0.7 dB/cm-MHz. We used a CH4-1 probe with excitation focal depth of 4.9 cm, transmit F/#2, and transmit frequency of 3.08 MHz. Both normalized cross correlation (NCC) and Bayesian displacement estimators were evaluated. We applied a quadratic motion filter to the data. To evaluate the error of the on-axis method as compared to traditional shear wave methods, we computed a robust lateral time-of-flight shear wave speed using a Radon sum transformation (LATSUM) and converted to a shear modulus for each phantom [3]. **Results and Conclusions:** The 15 phantoms had a mean shear modulus of 2.07 kPa and standard deviation of 0.12 kPa. We took the root mean square error of the shear modulus estimated using either the Bayesian displacement estimator or the NCC-derived estimator. In the depth of field, the median RMSE of shear modulus for the Bayesian estimator was 0.46 kPa and 0.93 kPa for NCC. The Bayes results show more agreement with the LATSUM results than NCC. These phantom results show that on-axis methods coupled with a Bayesian displacement estimator produce stiffness estimates comparable to laterally offset shear wave methods. [1] Sarvazyan, A. P., et al. "Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics." *UMB* 24.9: 1419-1435, (1998). [2] Walsh, K., et al. "On-axis radiation-force-based quantitative stiffness estimation with a Bayesian displacement estimator," *IEEE IUS*, 1-4, (2015). [3] Palmeri M. L., et al. "Quantifying Hepatic Shear Modulus In Vivo Using Acoustic Radiation Force," *UMB*, 34.4: 546-558, (2008).

112. Effects of Microenvironmental Mechanosensing on Cell Migration

Christopher Walter, Samila Nasrollahi, Amit Pathak

Biomedical Engineering

The ability of cells to communicate with their extracellular matrix (ECM) is important in many biological processes, including wound healing, development, immune response, and tumor metastasis. The mechanical properties that define the ECM such as stiffness, porosity, and geometry can all play major roles in controlling cell differentiation, shape, and migration. It has been shown that cell spreading depends on stiffness of the ECM away from the immediate microenvironment. However, this ability to sense distant ECM stiffness has been shown to be cell type dependent. Here, we cultured MCF10A human mammary epithelial cells on layered substrates, with a collagen layer of varying concentration and thickness attached on top of a polyacrylamide gel of defined stiffness. We tracked cell migration and performed morphological analysis of cells in contact with the collagen layer. We found that both the thickness and the concentration of collagen gels altered cellular response to polyacrylamide stiffness. We also found that focal adhesions and actomyosin contractility critically influence the ability of cells to sense both the micro-scale (collagen) and the macro-scale (polyacrylamide) ECM properties.

113. Quantitative Gas Transfer using Hyperpolarized ^{129}Xe MRI in Idiopathic Pulmonary Fibrosis(IPF)

Ziyi Wang, Scott H. Robertson, Jennifer Wang, Mu He et al

Duke University

ABSTRACT Background. Idiopathic Pulmonary Fibrosis (IPF) is an interstitial lung disease, in which progressive tissue fibrosis and inflammation impairs alveolar-capillary gas transfer. There is a pressing need to rapidly detect worsening function or therapy response of existing and new coming drugs. Here, we demonstrate novel methods to visualize and quantify ^{129}Xe uptake in barrier and RBCs to identify new biomarkers of gas exchange. Methods. The dissolved-phase images were decomposed into RBC and barrier images, which were then divided on a voxel-by-voxel basis by the gas-phase image to generate a gas transfer ratio map. These maps were denoised and displayed using linear binning (6 bins for RBC:gas map and 8 for barrier:gas map), with thresholds derived from 10 young healthy subjects (age: 27~31). This mapping was then applied to 12 IPF patients, 7 of whom had follow-up scans to assess progression. Results. Compared to healthy subjects, IPF patients show regions of diminished RBC:gas and dramatically higher barrier:gas. In IPF patients, significantly more voxels fell in the lowest two RBC:gas bins ($p < 0.001$, $p < 0.001$), while considerably more voxels fell in the 3 highest barrier:gas bins ($p < 0.001$, $p < 0.001$, $p < 0.001$). Preliminary evaluation of ratio map and histogram analysis shows the ability to detect disease progression and possible therapy response. Conclusions. Quantitative analysis method using binning maps with thresholds derived from a reference population reveals the key features of IPF. IPF is characterized by significantly enhanced barrier:gas intensity throughout the lung whereas RBC:gas remains normal over much of the lung. This quantitative binning maps of ^{129}Xe gas exchange are likely to be useful for assessing numerous other disorders.

114. Imaging Bacterial Infection with ^{68}Ga -[^{18}F]-Fluoromaltotriose and Positron Emission Tomography

Mirwais Wardak, Gayatri Gowrishankar, Evgenios Neofytou, Mohammad Namavari et al
Stanford University

Purpose: Bacterial infections continue to represent a significant cause of morbidity and mortality worldwide, especially with the emergence of several strains of resistant pathogenic bacteria (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA], multidrug-resistant *Streptococcus pneumoniae*, etc.). The current gold standard for the diagnosis of bacterial infections is based on the examination and culture of bacteria recovered from suspected sites. However, this method is invasive, time-consuming, and unable to determine the spread of infection. Moreover, it is important to find a method that can distinguish bacterial infection from non-bacterial inflammation. Recently, we developed a novel positron emission tomography (PET) tracer called ^{68}Ga -[^{18}F]fluoromaltotriose which is transported via the maltose transporter, a transport system that is exclusive to bacteria and not expressed on mammalian cells. The purpose of this study was to investigate ^{68}Ga -[^{18}F]fluoromaltotriose PET imaging for the detection, quantification, and therapeutic monitoring of bacterial infections in-vivo. **Methods:** Three nude rats, which contracted a visceral staph infection post-cardiac surgery, had dynamic microPET scans performed on them with the tracer ^{68}Ga -[^{18}F]fluoromaltotriose before and after 1-month of therapy with Cefazolin (an antibiotic). List-mode PET data were acquired for 40 min (28 frames total) on a small-animal Inveon microPET/CT scanner immediately after tracer injection via a tail vein catheter. A healthy, immunocompetent rat with an intact immune system served as our control. The longitudinal PET images were visually and quantitatively assessed. **Results:** At baseline before the start of antibiotic treatment, the ^{68}Ga -[^{18}F]fluoromaltotriose PET images showed high tracer uptake at likely sites of bacterial infection near the heart. The tracer did not accumulate in inflamed tissue and had a good clearance profile. In addition, the microflora in the gut is seen with the tracer. On post-therapy PET images, the original sites of bacterial infection were significantly decreased in their tracer uptake, indicating therapeutic response. The PET image of the control rat did not show any significant signal in the myocardial region as expected. **Conclusions:** ^{68}Ga -[^{18}F]Fluoromaltotriose can be used to image and monitor bacterial infections in-vivo with high sensitivity and specificity. We believe that this class of imaging probes will have a significant impact on the clinical management of patients suspected of having bacterial infections. Based on these preliminary results, plans are being made to do more animal studies and then bring this radiotracer to human trials.

115. Multimodal Neuroimaging Evaluation of the Default Mode Network in Chronic Traumatic Brain Injury

Jeffrey Ware,

University of Pennsylvania, Department of Radiology

Purpose Traumatic brain injury (TBI) is a leading cause of cognitive morbidity around the world, for which functional outcomes have improved little over time. Difficulties in developing more effective rehabilitation strategies stem in part from incomplete understanding of the neurobiological substrates of cognitive disability following TBI. While existing evidence suggests that structural, functional, and metabolic alterations within the brain's default mode network (DMN) may underlie specific cognitive sequelae of TBI, few studies to date have employed multimodal approaches to concurrently investigate these relationships. In this study, a multimodal approach is used to characterize functional, perfusional, and structural abnormalities of the DMN in relation to posttraumatic cognitive deficits. **Methods** This study includes 43 subjects who sustained TBI of at least moderate severity and 35 demographically-matched healthy controls. Neuropsychological and neuroimaging evaluation was performed at 3 months following injury in the TBI group. Imaging consisted of a magnetization-prepared recalled-gradient echo (MPRAGE) T1-weighted sequence, 2D pseudo-continuous arterial spin-labelling (pCASL) brain perfusion, and a 10-minute resting state fMRI acquisition. T1-weighted images were used for manual segmentation of macrostructural lesions and assessment of atrophy using a tensor-based morphometry (TBM) approach. ASL data were used to derive whole-brain maps of CBF. Resting state data were used to derive measures of static and dynamic resting state functional connectivity (FNC) within the DMN. Initially, whole-brain CBF and measures of structural atrophy were compared in between TBI and control groups. Subsequent analysis focused specifically on the relationship between neuropsychological measures and DMN-specific CBF, functional connectivity, and atrophy. **Results** Compared to healthy controls, subjects with TBI demonstrated several regions of significantly reduced resting perfusion in both cortical and subcortical locations ($p < 0.01$). Reduced frontal and temporal cortical perfusion demonstrated spatial correspondence with encephalomalacia. Reduced CBF was also present in locations without corresponding macrostructural lesions such as the thalamus and sub-regions of the DMN, which also demonstrated evidence of atrophy. Relative to controls, subjects with TBI had elevated static FNC and reduced dynamic FNC within the DMN. Among the TBI subject group, DMN CBF correlated directly with attentional function ($r = 0.37$, $p = 0.01$) and inversely with dynamic connectivity ($r = -0.35$, $p = 0.02$). **Conclusions** Chronic TBI is associated with persistent and inter-related alterations in structural integrity, CBF, and functional connectivity of the DMN. Our findings suggest that DMN abnormalities are closely related to posttraumatic attentional dysfunction, and demonstrate the advantages of using multimodal neuroimaging approaches to characterize and better understand the neurobiological sequelae of TBI.

116. Discovery of Novel Modulators of the $\alpha 3$ Glycine Receptor

Marta Wells, Yan Xu, Pei Tang

University of Pittsburgh

Purpose – Glycine receptors (GlyRs) are inhibitory chloride-selective pentameric ligand-gated ion channels found primarily in the brainstem and spinal cord. $\Delta 9$ -tetrahydrocannabinol (THC) potentiates GlyR- $\alpha 1$ and GlyR- $\alpha 3$ subtypes through allosteric interactions with residue S296 in the transmembrane domain of the receptor. This positive modulation directly contributes to cannabis-induced analgesia and is independent of the other psychoactive effects of THC. Here, we perform virtual screening at the S296 cannabinoid-binding site on an ensemble of GlyR- $\alpha 3$ structures, in vitro functional validation of top ranked compounds, and subsequent molecular dynamics simulations to characterize novel modulators of GlyR- $\alpha 3$. **Methods** – The transmembrane domain of the antagonist-bound GlyR- $\alpha 3$ crystal structure (PDB ID: 5CFB) and a homology model of the open state GlyR- $\alpha 1$ NMR structure (PDB ID: 2M6I) were used as independent starting points for molecular dynamics simulations to obtain a diverse ensemble of GlyR- $\alpha 3$ structures. Over 2 million compounds from the ZINC database of drug-like molecules were screened at the S296 cannabinoid-binding site on each receptor structure. Screened compounds were pre-filtered by physicochemical features selected for their ability to penetrate the blood-brain barrier and affect the central nervous system. Drugs were ranked based on their predicted binding affinities across GlyR- $\alpha 3$ structures in the closed and open states. **Results** – Leading compounds were selected for experimental validation in *Xenopus laevis* oocytes expressing human GlyR- $\alpha 3$. Several top ranked compounds were found to exhibit dose-dependent potentiation, while others inhibited glycinergic currents. The two most potent novel potentiators and inhibitors, respectively, were selected for further characterization in molecular dynamics simulations. All four compounds were shown to be stable at the S296 cannabinoid binding site, and simulations revealed specific interactions involved in the binding of potentiators and inhibitors and in their mechanisms of allosteric modulation. **Conclusions** – We provide compelling evidence that these novel potentiators may be as effective as THC in relieving pain by modulating GlyR- $\alpha 3$. The identified compounds are strong candidates for further evaluation of their therapeutic potential in in vivo experiments. In addition, we have shown that interactions at the S296 cannabinoid-binding site on GlyR- $\alpha 3$ can produce both positive and negative allosteric effects, demonstrating the complexity of the molecular mechanisms of drug modulation in glycine receptors.

117. In Vivo Monocyte Tracking by PET and Fluorescence

Moses Q. Wilks, Marc D. Normandin, Hushan Yuan, Charalambos Kaittanis et al

Massachusetts General Hospital

Purpose: Immune system response, specifically monocyte and macrophage infiltration, is an important component in a wide range of diseases, with many ongoing investigations and clinical trials to measure this response. Here we aim to develop a method to monitor monocyte/macrophage trafficking with increased sensitivity, by loading cells both in and ex vivo with a radio-labeled nanoparticle for PET imaging. **Methods:** Monocytes and macrophages were labeled using a modified form of the FDA approved drug Feraheme (FH), a treatment for iron anemia and a strong MRI contrast agent. This compound was modified with the addition of ^{89}Zr for PET imaging and/or fluorochrome (Cy5.5) for microscopy and cytometry. **In Vitro:** Whole blood and isolated white blood cell fractions were taken from multiple species (mice, non-human primate, and pig), and were incubated with fluorescent and radioactive versions of the imaging agent, at multiple drug concentrations and incubation temperatures, for variable times. Cellular uptake of nanoparticle was measured by relaxometry, gamma counting, and flow cytometry. **In Vivo:** Mice were given one IV injection of ~ 250 uCi of ^{89}Zr -FH, and imaged serially by microPET/CT for up to two weeks. Additionally, Cy5.5-FH was administered IV to non-human primates. Blood samples were taken from these animals up to 5 days after administration, and cell labeling was measured by flow cytometry. **Results:** In vitro incubation across modalities showed time and concentration dependent labeling of the white blood cell fraction, with labeling blocked when incubated at 4°C . Flow cytometry showed that monocytes and macrophages were specifically labeled by Cy5.5-FH while all other cell types remained unlabeled. PET results showed high ^{89}Zr -FH uptake in the liver and spleen, with standardized uptake values (SUV) in these organs of ~ 10 . There were also high levels of ^{89}Zr -FH in the lymphatic system, with SUVs in excess of 20-30 in some lymph nodes. Blood clearance half-life of the compound was $\sim 1\text{h}$, but the uptake processes into the lymph nodes had half-lives ranging between 12-24 hours, suggesting a slow cell trafficking process. This was confirmed by FACS analysis of primate blood taken after administration of Cy5.5-FH. Peak monocyte labeling occurred approximately 3 hours after injection, but slow cell trafficking of these cells out of the blood pool was observed, with labeled circulating monocytes present up to 5 days after administration. **Conclusion:** We showed a method to specifically label monocytes with a radio-labeled nanoparticle, allowing for sensitive and quantitative measurement of monocyte trafficking in vivo.

118. Localized, Gradient-reversed Ultrafast Z-spectroscopy in vivo at 7T

Neil Wilson, Kevin D'Aquila, Catherine Debrosse, Hari Hariharan et al

University of Pennsylvania

Purpose: Chemical exchange saturation transfer is a highly sensitive MR technique for observing metabolite content where labile protons are selectively saturated and allowed to exchange with water protons. A z-spectrum is plot of the saturation as a function of offset frequency. Here, we developed a technique to collect ultrafast z-spectra in vivo that is relatively robust to voxel inhomogeneity. **Theory:** Saturating in the presence of a gradient encodes the frequency offset spatially across a voxel. This encoding can be resolved by applying a similar gradient during readout. Acquiring additional scans with the gradient polarity reversed effectively mirrors the spatial locations of the frequency offsets so that the same physical location of a positive offset in the original scan will contribute a negative offset in the gradient-reversed scan. **Methods:** Gradient-reversed ultrafast z-spectroscopy (GRUFZS) was implemented and tested in a modified, localized PRESS sequence at 7T. Lysine phantoms were scanned at various concentrations and compared with coventionally-acquired z-spectra. Scans were acquired in vivo in human brain in homogeneous and inhomogeneous voxels with the ultrafast direction cycled between r, p, and s. Results were compared to those from a similar conventional z-spectroscopy PRESS-based sequence. **Results:** Asymmetry spectra from GRUFZS are more consistent and reliable than those without gradient reversal and are comparable to those from conventional z-spectroscopy. GRUFZS offers significant acceleration in data acquisition compared to traditional CEST methods with high spectral resolution and showed higher relative SNR efficiency. **Conclusion:** GRUFZS offers a method of collecting ultrafast z-spectra in voxels with the inhomogeneity often found in vivo.

119. Functional Connectivity Structure of Cortical Calcium Dynamics in Anesthetized and Awake Mice

Patrick W. Wright, Adam Q. Bauer, Grant Baxter, Matt D. Reisman et al

Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO 63130

Purpose: Hemodynamic-based markers of cortical activity (e.g. fMRI, optical intrinsic signal imaging), driven by electrical and metabolic activity through neurovascular coupling, are an indirect and slow report of brain function and are limited in their utility to deduce underlying brain network dynamics. Here we extend functional connectivity (FC) analysis, a method for mapping functional relationships using spontaneous brain activity, from hemodynamic to Ca²⁺-dynamic imaging. Methods: Transgenic mice (n=7) expressing a fluorescent calcium indicator (GCAMP6) driven by the Thy1 promoter in cortical glutamatergic neurons were imaged transcranially in both ketamine-anesthetized and awake states. Sequential LED illumination ($\lambda=470, 530, 590, 625\text{nm}$) enabled concurrent imaging of both GCAMP6 fluorescence emission (corrected for hemoglobin absorption) and hemodynamic activity. Somatosensory responses were evoked using a 0.5mA electrical hindpaw block paradigm. Correlative FC network maps were generated for low (0.009-0.08Hz) and high (0.4-4Hz) frequency bands. Cross-correlation analysis was used to calculate time delays between GCAMP6 and HbO₂ evoked responses as well as to construct pixelwise delay maps between the time series of spontaneous activity at each pixel relative to the whole-brain signal. Results: Following hindpaw stimulation, GCAMP6 provided a response time course sensitive to individual high frequency (2Hz) pulse presentations and preceded the stereotypical hemodynamic response function by $\sim 0.65\text{s}$. Homotopic HbO₂ and GCAMP6 FC maps have similar topographies at low frequencies. At higher frequencies, GCAMP6 is sensitive to delta band (0-4Hz) activity associated with slow-wave sleep. This phenomenon provides a striking effect on the correlation structure of the FC maps from anesthetized mice that is diminished upon wakefulness. This state-dependent contrast is driven by an anterior-posterior delay topology associated with delta band activity. Conclusions: In summary, functional neuroimaging of Ca²⁺ dynamics in mice provides evidence that spatiotemporal coherence in cortical activity is not exclusive to hemodynamics. Concurrent Ca²⁺ and hemodynamic-based imaging will enable the dissociation of changes in ionic networks, hemodynamic networks, and neurovascular coupling and provide a framework for subsequent studies of neurological disease, such as stroke.

120. Textile Platform for Musculoskeletal Tissue Engineering

Iman K. Yazdi, Afsoon Fallahi, Huseyin Avci, Ali Tamayol et al

Harvard Medical School, Brigham and Women's Hospital

Introduction: Designing constructs that can mimic native skeletal muscle and induce 3D cellular alignment and elongated myotube formation remains an ongoing challenge for skeletal muscle tissue engineering. Textile platform have opened a new area in the field. Precise control over the distribution of different cell types and microarchitecture of fabricated constructs are considered as key advantages of this technology. Insufficient mechanical properties of cell-carrying hydrogel fibers have limited their use. Thus, the concept of composite cell-laden fibers that can tolerate textile processing and support long-term cell survival and functionality has been recently explored to address these challenges. In this work, we present core–shell composite fibers comprising a biodegradable core and UV crosslinkable hydrogel shell. Alginate (ALG) and methacrylated gelatin (GelMA) were blended together to prepare composite hydrogel shell. While ALG offers a good mechanical support and a template to control cellular alignment, GelMA mimics the extracellular matrix and support cellular proliferation and differentiation, desired cell functions, and interactions. **Materials and Methods:** Composite cell-laden fibers were fabricated by coating a hydrogel blend mixed with cells on a biodegradable collagen based suture from ALG-GelMA with concentrations of 1-2% and 10% w/v, respectively. A cell-laden hydrogel fiber was prepared by passing a thread through a hollow channel filled with ALG-GelMA solution mixed with C2C12 cells and then crosslinked with 2% (w/v) CaCl₂ to strengthen ALG network and then GelMA was further crosslinked through UV irradiation (365 nm, 850 mW). We performed physical and mechanical characterization of composite fibers including compressive and elastic modulus. In addition, we assessed viability and metabolic activity of encapsulated cells using live/dead and PrestoBlue assays. Cellular morphology and alignment were also assessed through microscopy using α -actin and nuclei immunostaining. **Results and Discussion:** Fabricated composite cell-laden fibers containing C2C12 has a thickness of 700 to 1200 μ m by adjusting the diameter of hollow channel. Encapsulated cells in 1 and 1.5% ALG concentration in ALG-GelMA composites appeared to have higher survival and bioactivity compared to 2%. According to these findings, at lower ALG concentration higher cell viability and adhesion were seen over 7 days of culture. In addition, immunostaining data demonstrated that at higher ALG concentration, lower cell elongation behavior was seen. Furthermore, the cell alignment behavior appeared to be directly in correlation with cell density and the diameter of fiber. **Conclusions:** We developed a simple technique to fabricate composite cell-laden hydrogel fibers composed of a biodegradable core and a photocurable hydrogel shell for mimicking the native skeletal muscle tissue. Encapsulation of C2C12 myoblasts in these fibers demonstrated high viability and ability to induce cellular alignment and elongation. These results suggest that by encapsulating cells into composite hydrogel, fiber structure guides cellular alignment and elongated myotube formation and provides a suitable 3D environment for nutrition exchange and mechanical support, which can potentially be used for skeletal muscle regeneration applications.

121. Genetically Engineered Human Induced Pluripotent Stem Cells to Model Cronos Titin

Rebecca Zaunbrecher, Shiv Bhandari, Andrea Leonard, Kevin Beussman et al

University of Washington

The giant protein titin has numerous important roles in the cardiomyocyte, including providing passive tension and facilitating sarcomere formation. Recently an internal promoter was identified in the titin gene (TTN) indicating the presence of a previously unstudied isoform, Cronos. Although the function of Cronos titin is unknown, the majority of disease-causing mutations in TTN are found downstream of this internal promoter suggesting an important role in health and disease. To create a cell line to study the role of Cronos titin we have introduced homozygous frameshift mutations in exon 2 of the TTN gene in human induced pluripotent stem cells (Ex2 KO hiPSCs) using the CRISPR/Cas9 system to prevent translation of full-length titin while leaving the Cronos isoform intact. Directed differentiation of Ex2 KO hiPSCs into cardiomyocytes (Ex2 KO hiPSC-CMs) yields contracting cells, and immunofluorescence studies indicate the formation of short, dispersed myofibrils in Ex2 KO hiPSC-CMs compared to isogenic wildtype controls. Additionally, staining Ex2 KO hiPSC-CMs with antibodies specific to the MIR and M8-M10 regions of titin downstream of the Cronos internal promoter demonstrates incorporation of these domains into the sarcomere. Staining of Ex2 KO hiPSC-CMs with antibodies specific to the Z1Z2 and PEVK regions of titin upstream of the internal Cronos promoter suggests these domains are not present in the sarcomeres. We conclude that the Ex2 KO hiPSC-CMs present a system in which to study Cronos titin and could provide important insights into the role of this isoform in sarcomere formation and function.

122. Investigating Population Structure in the *Drosophila* Genome Nexus

Roy N. Zhao, J.J. Emerson

University of California, Irvine

The *Drosophila* Genome Nexus (DGN) dataset represents an extremely versatile resource for population genetic and genomic research in *D. melanogaster*, comprising a collection of hundreds of genome sequences generated under a consistent and rigorous protocol. When performing genetic analyses, it is useful to consider the population structure that exists within a dataset, as it can confound conclusions about topics of interest such as nucleotide diversity, natural selection, and allele frequency distributions. Population structure within the DGN genomes was investigated using the software 'structure,' and a parallel-processing framework for 'structure' was developed to efficiently explore large datasets concurrently. Analyses performed on the DPGP3 subset comprising 197 genomes from a single sub-Saharan ancestral range population reflect potential internal demographies within the sample. Population structure inferences for the DPGP3 data indicated groupings that were not reported in the initial publication of the DGN, with several of the genomes being reliably assigned to common clusters over multiple runs. The preliminary results suggest the potential to detect fine-scale population structure within single ancestral range *D. melanogaster* populations, which are free from out-of-Africa bottleneck effects and mostly unaffected by cosmopolitan admixture. Applying thorough population structure analyses to large, diverse, and methodologically consistent datasets like the DGN can facilitate the discovery of population genetic insights with high resolution, less bias, and higher power. Additionally, the parallelized version of 'structure' is a broadly useful tool, expanding the software's usefulness for analyzing large genomic datasets and enabling its flexible deployment on high-performance computing systems.