**Practical Surgical Planning Toolbox for Convection-Enhanced Delivery in Non-Human Primate Brains**

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Non-human primate (NHP) research has become an essential step in the translation of medical technologies from animal models to clinical trials. This is especially so in neural research, as there is a large discrepancy between rodent and human brains in both anatomy and size. For some techniques such as optogenetics, which requires viral transduction of neurons, traditional diffusion-based viral infusion approaches are effective in rodent brains but are impractical for large NHP brains. Convection-enhanced delivery (CED), a large-scale infusion approach, currently lacks a practical quantitative bench-side infusion modeling method to guide neurosurgical preparation. To mitigate surgical risk, we have developed a gel model of the NHP brain and tested the validity of our model by monitoring the spread of the infusion through the gel and comparing the data with those from MRI scans of previous infusions in NHP. Since CED can behave differently depending on the location of infusion in the brain, we tested bench-side infusions at different depths to validate the versatility of our model and demonstrated that our model is applicable to these different depths. Additionally, we are developing methods of reducing tissue damage caused by the cannula insertion by testing the effectiveness of smaller cannula sizes with our bench-side model. We found that some cannula designs are able to maintain the same infusion results as our traditional, larger cannulas and we plan to perform CED infusions in rat brains with these new cannulas to quantify tissue damage reduction.

**4D Control of Protein Photoactivation in Hydrogel Biomaterials to Guide Stem Cell Fate**

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Life depends on a series of well-orchestrated biochemical reactions facilitated by proteins, which are differentially transcribed and activated in response to changing conditions. Hydrogels, water-swollen polymeric biomaterials, have proven useful as synthetic platforms to probe and direct biological activities by enabling researchers to recapitulate many aspects of the native cell environment. Though current hydrogel protein patterning techniques are capable of driving specific cell fates in individual cells in time and space (i.e. 4D), the timescales for patterning place dramatic limits on the types of biological functions that can be controlled. Furthermore, current techniques rely on slowly diffusing bioactive proteins into materials prior to immobilization within gels, so complete temporal control of protein activation within hydrogels remains out of reach. To address these limitations, my project focuses on directly photoactivating proteins within hydrogels using cytocompatible light. We predict that the extent of protein activation can be controlled dose-dependently by varying light exposure duration and intensity. We intend to use this platform to direct stem cell migration, differentiation, and proliferation in 4D on physiologically relevant timescales, which has tremendous utility in stem cell biology and regenerative medicine.

**Osmotic Processor for Enabling Sensitive and Rapid Biomarker Detection via Lateral Flow Assays**

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Urine is an attractive biospecimen for in vitro diagnostics, and urine-based lateral flow assays are low-cost devices suitable for point-of-care testing, particularly in low-resource settings. However, some of the lateral flow assays exhibit limited diagnostic utility because the urinary biomarker concentration is significantly lower than the assay detection limit, which compromises the sensitivity. To address the challenge, we developed an osmotic processor that statically and spontaneously concentrated biomarkers. The specimen in the device interfaces with the aqueous polymer solution via a dialysis membrane. The polymer solution induces an osmotic pressure difference that extracts water from the specimen, while the membrane retains the biomarkers. The evaluation demonstrated that osmosis induced by various water-soluble polymers efficiently extracted water from the specimens, ca. 5 – 15 mL/hr. The osmotic processor concentrated the specimens to improve the lateral flow assays’ detection limits for the model analytes—human chorionic gonadotropin and SARS-CoV-2 nucleocapsid protein. After the treatment via the osmotic processor, the lateral flow assays detected the corresponding biomarkers in the concentrated specimens. The test band intensities of the assays with the concentrated specimens were very similar to the reference assays with 100-fold concentrations. The mass spectrometry analysis estimated the SARS-CoV-2 nucleocapsid protein concentration increased ca. 200-fold after the osmosis. With its simplicity and flexibility, this device demonstrates a great potential to be utilized in conjunction with the existing lateral flow assays for enabling highly sensitive detection of dilute target analytes in urine.

**CryoGrid-PIXUL-Matrix System to Interrogate Intratumor Epigenetic Heterogeneity of MGMT Gene in Glioblastoma**

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Glioblastoma multiforme (GBM) is known as the most aggressive brain tumor and almost always lethal. The DNA methylation of O6-methylguanine-DNA methyltransferase (MGMT) promoter gene has been extensively used as a GBM biomarker to predict prognosis and stratify patients for treatments. Previous research has shown that GBM features exhibit significant molecular heterogeneity within a tumor, which has forced the utilization of multiple, rather than single, biopsies to optimize potential therapies. Therefore, the goal of this project is to define the scope of intratumor heterogeneity of MGMT methylation in GBM and its relation to tumor histology to determine sampling biopsy needs. The lab has developed high throughput systems to preserve and sample tissue in frozen state using CryoGrid-CryoCore system and to prepare DNA using PIXUL multi-sample sonicator. To assay epigenetic markers, including DNA-methylation, the system incorporates matrix methylated DNA immunoprecipitation (MeDIP) and quantitative PCR (qPCR). We sectioned dozens of frozen GBM tumors and use CryoGrid-PIXUL-Matrix-MeDIP-qPCR system to assess intratumor MGMT methylation heterogeneity. Thus far, our results suggest that low level of intratumor MGMT methylation heterogeneity and sampling GBM tumor in three different regions will provide clinicians with reliable information regarding prognosis and treatment of this deadly cancer.

**The IL-2/IL-15 Mimetic NL-201 Prevents Myeloma Relapse after ASCT by Expanding Highly Cytolytic T Cells in the Bone Marrow**

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Multiple myeloma (MM) is a bone marrow (BM) resident hematological malignancy that is becoming increasingly recognized as one amenable to immunotherapy. We have established that addition of T cells to BM grafts enhanced myeloma control post-autologous stem cell transplant (ASCT) in mice. Approaches aimed at improving T cell responses post-ASCT may therefore prove highly effective. To explore this, we utilized the interleukin-2 (IL-2)/interleukin-15 (IL-15) mimetic NL-201: a de novo cytokine mimetic that signals via the IL-2 receptor beta and gamma chain (IL-2Rβ/IL-2Rγ) subunits without engaging the IL-2 receptor alpha chain (IL-2Rα). IL-2Rα signaling has been associated with IL-2-mediated toxicity. We hypothesized that NL-201 would enhance control of myeloma progression by stimulating T cell proliferation and activation early post-ASCT. We transplanted irradiated myeloma-bearing recipients with a BM and T cell graft and administered NL-201 post-ASCT. NL-201 promoted potent anti-myeloma immunity that was dependent on CD4 and CD8 T cells, but not natural killer cells. To further elucidate potential mechanisms of action we harvested BM from vehicle and NL-201-treated mice and performed comprehensive immunophenotyping with high parameter flow cytometry. Mechanistically, NL-201 significantly expanded the total number of CD8 T cells in the BM. Memory CD8 T cells were preferentially expanded, while the frequency of exhausted CD8 T cells was reduced in NL-201-treated mice compared to vehicle-treated mice. Surprisingly, a larger percent of memory CD8 T cells in NL-201-treated mice produced granzyme B compared to vehicle-treated mice. Granzyme B production was also observed in conventional CD4 T cells in response to NL-201 treatment, and the frequency of regulatory T cells was reduced by 50% in NL-201-treated mice. NL-201 expanded bone marrow resident cytotoxic memory CD8 and CD4 T cells without driving T cell exhaustion, whilst reducing the frequency of regulatory T cells in the BM tumor microenvironment. Together, these
data highlight the promising therapeutic potential of NL-201 post-transplant in multiple myeloma.

**Discovery of CD28 Aptamers for T-Cell Activation and Immune Modulation**

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The CD28 receptor provides co-stimulatory signaling as part of T-cell activation, thereby driving T-cell proliferation, differentiation, cytokine production, and survival required for effective immune responses. Given this important role, CD28 has broad therapeutic implications, serving as a target for cancer immunotherapy, treatment of autoimmune disorders, and production of adoptively transferred T cells. Current approaches for targeting CD28 rely on antibodies, which can be employed in vivo or ex vivo to promote or block CD28 signaling depending on the application. While effective, antibody-based targeting is costly and rigid in design, owing to their biological production and reduced control over binding. Aptamers are small, single-stranded oligonucleotides with sequence-defined architectures that can bind specific targets of interest at high specificity and affinity. Aptamers can be produced at low cost and the inherent properties of oligonucleotides permit flexibility in reversing binding and fine-tuning affinity strength for optimal receptor targeting. This project proposes to develop the first aptamer that targets human CD28 using a combinatorial selection strategy that incorporates protein- and cell-based selections. Aptamer candidates will be identified and characterized to evaluate their binding specificities and kinetics. The selected aptamer will then be used to design a T cell activation assay. A 12-round selection has been completed and binding specificities of individual aptamer candidates will be evaluated. A second selection using a modified approach is currently in progress. If successful, this project has the potential to improve the T-cell activation process in manufacturing adoptive T cell therapies and facilitate the development of novel therapeutics for treating cancer and autoimmune diseases.