The Effect of Holliday Junction Isomeric Form and Sequence on the Self-assembly of Rationally Designed DNA Crystals

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The majority of protein crystal structures to date have been determined via X-ray crystallography. However, producing high resolution crystals has proven to be a major obstacle for structural analysis for hundreds of proteins which rely upon arbitrary crystallization screens with severely limited predictability. Our goal is to rationally design 3D DNA crystals *a priori*, with a fully addressable interconnected network of helical arrays that can immobilize guest molecules at specified positions, to allow their structure to be solved without having to find suitable crystallization conditions. DNA nanotechnology has emerged as a prominent field that uses DNA as a molecular scaffold for programmable self-assembly of crystalline arrays in both 2D and 3D.[1,2] The central building block of structural DNA nanotechnology is an immobile Holliday junction, a branched nucleic acid motif inspired by the structure formed during homologous recombination. DNA-directed crystal design introduces single-stranded overhangs (“sticky ends”) into these branched four arm junctions, allowing them to self-assemble sequence specifically via canonical Watson-Crick base pairing. Previously, our lab has determined that junction sequence of the DNA crystals affects their crystallization capability, structure, and symmetry.[3] Here, we probe the effect of the alternative isomer (“Isomer II”) of 36 unique Holliday junction sequences. Thus far, we show that Isomer II can rescue junction sequences previously deemed “fatal” for crystallization and affect crystal symmetry. This work will inform the improved design and crystallization of self-assembled DNA crystals, enabling the scaffolding of biomolecules for the first ever use of a DNA crystalline lattice to aid structure determination.

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2. Wilner, O. et al. Nat. Nanotechnol. 4, 249-254 (2009).

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Title: Sex-dependent circulating sex hormone response to traumatic brain injury and implications for drug delivery to the injured brain

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Abstract: Traumatic brain injury (TBI) refers to the damage and impairment of the brain following an external blow or jolt to the head. TBI pathology includes acute and chronic phases that are classified by their effects on the patient. The acute phase consists of the immediate injury-induced neurological damage that transitions to a chronic phase of secondary events, including the dysfunction of the blood-brain barrier (BBB) that regulates transport of substances into and out of the brain. Current research focuses on using a variety of methods to stimulate the restoration of neural function post-injury. Our lab explores the delivery of nanoparticles (NPs) to effectively transport drugs across the BBB, which is made possible due to the permeability of the BBB for up to 7 days after injury. We have previously shown a fundamental difference in NP delivery and accumulation between male and female mice following TBI, suggesting that mechanisms related to biological sex influence BBB permeability and subsequent NP extravasation profiles. Here, we focused on characterizing the circulating sex hormones following TBI (24hr post-injury) to eventually determine the impact on BBB dysfunction. We used a well-established mouse controlled cortical impact model to induce a moderate/severe focal TBI. Prior to injury, females were estrus cycle tracked using vaginal smears (n = 12 per group) to record the cycle phase at injury and sacrifice. At 24hrs post-injury (injured groups), plasma samples were collected at sacrifice and analyzed for testosterone, androstenedione, estradiol, estrone, progesterone, and corticosterone via mass spectrometry. Interestingly, within this hormone profile 24hrs post-injury, injured females had significantly lower levels of androstenedione compared to naïve females. Androstenedione is an androgen steroid hormone precursor to estrone, estradiol and testosterone. Additional analyses include RNA-sequencing and immunohistochemical analysis of tight junctions and adherens junctions. Subsequent time points post-injury will also be completed characterize the temporal profile of circulating sex hormones post-injury. Together these data will determine how/if biological sex and circulating sex hormones impact BBB permeability after TBI. The results of these experiments will determine the most opportune time to deliver nanoparticles post-injury in male and female mice. Ultimately, we hope these experiments will create more-personalized therapeutics that promote neural regeneration after TBI.

Title: Design and characterization of multifunctional polymeric nanoparticles for traumatic brain injury

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Traumatic brain injury (TBI) is a serious health concern that poses an enormous economic burden to the healthcare system and can result in long term disabilities. TBI is a heterogeneous disease that triggers series of complex signaling cascades beginning shortly after insult and continue developing over months involving metabolic dysfunction, excitotoxicity, neuroinflammation, autoimmunity, apoptosis, neurodegeneration, and blood-brain barrier (BBB) disruption. In a healthy brain the BBB prevents the entrance of foreign substances, but unfortunately, it also prevents the entry of therapeutic agents. After trauma, the BBB becomes semi-permeable providing a therapeutic window to deliver drugs directly to the brain and develop effective treatments. Taking advantage of this opportunity, we designed a homing polymeric nanoparticle (NP) with the capability of encapsulating and delivering a drug at a specific timepoint after the injury. The NPs are being synthetized using poly(lactide)-block-poly(ethylene glycol) with two different chemical handles that allow biorthogonal conjugation of the homing peptide and a fluorescent dye. The homing peptide is highly specific to the injury penumbra 24 hours after injury. This design allows for the delivery of pharmaceutics that are tailored to treat the injury at specific time post-injury. Ongoing work will corroborate the feasibility of the proposed NP and the efficacy of the targeting peptide.

CAR-Macrophages based therapy as a treatment for human ovarian cancer

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Ovarian cancer ranks fifth in terms of death caused by cancer. Furthermore, the only available option is generic therapeutics which are not very effective. CAR or Chimeric antigen receptor strategy involves generating a receptor against the cancer-specific antigens. Although CAR-T therapy has become extremely popular in the past few years, they suffer from drawbacks like their inability to infiltrate solid tumours like ovarian cancer. Macrophages can be a better alternative to T cells since they are constantly being recruited to the tumour microenvironment. Ovarian cancer cells are known to over-express the mesothelin protein and therefore, in this project, we propose to generate CAR- Macrophages expressing mesothelin scFv as a therapy against ovarian cancer cells.

Methods THP1 cells or human monocyte cell line and Ovarian cancer cell line were used for this study. Transfection of mesothelin scFv mRNA and plasmid was performed using lipofectamine-3000. Transfection efficiency was measured using flow cytometry and ELISA. The ability of the CAR-cells to interact with rhodamine-stained Ovarian cancer cells was measured after performing a co-culture assay and performing flow cytometry. This was followed by in-vivo experiments in which the CAR-cells were injected into mice containing Ovcar8 tumours.

Results: *In-vitro* studies of transfection of THP1 cells with mesothelin scFv have shown a noticeable increase in GFP expression, correlating to successful transfection, in mRNA and plasmid transfected samples compared to the controls. Additionally, the phagocytosis exhibited was seen to be higher in plasmid- transfected samples. This elucidates that CAR-Macrophages can be considered as a potential therapeutic against ovarian cancer.

Development and characterization of injectable, guest-host hydrogels for neural tissue engineering applications

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INTRODUCTION: Traumatic brain injury (TBI) is a global health concern that affects millions of people each year. Primary and secondary injury sequences associated with a sustained TBI lead to acute and chronic cellular processes that exacerbate the damage caused by the initial injury. While current research focuses on the use of tissue engineering strategies to modulate the injury pathology, there are no approved therapies to treat the underlying cellular mechanisms involved in the progression of TBI-related tissue damage. Tissue engineering approaches use a combination of cells, signaling molecules, and biomaterials to facilitate repair and regeneration. A hyaluronic acid (HA)-based hydrogel that is crosslinked via hydrophobic interactions between adamantane groups and cyclodextrin groups that combine to form a guest-host complex in solution was chosen for this study. The reversible nature of guest-host crosslinks provides the hydrogel with shear-thinning and self-healing capabilities that allow it to be injected into the damaged tissue with minimal diffusion and ejection. In this study, we synthesize and characterize the guest-host hydrogel for a novel application in the treatment of traumatic brain injury.

METHODS: Adamantane (guest group) and cyclodextrin (host group) modifications of HA were performed through esterification and amidation reactions, respectively. The cyclodextrin-HA was further modified with a methacrylate group to facilitate the covalent attachment of the cell adhesion motifs, RGD and IKVAV, through Michael-type addition reactions. Tethering of the cell adhesion peptides was confirmed using a bicinchoninic acid (BCA) assay to determine the presence of peptide in a purified sample. The guest and host-modified HA polymers were then combined in aqueous solution to form a hydrogel. Rheology was performed on hydrogels of varying HA weight percentage to determine the appropriate formulation for central nervous system (CNS) tissue. Afterwards, a lactate dehydrogenase (LDH) assay was used to assess the cytotoxicity of the guest-host hydrogels towards primary mouse astrocytes after 24 hours of exposure. The hydrogel formulation determined to be ideal for CNS tissue applications will be used for subsequent in vivo biocompatibility studies in a mouse model of TBI.

RESULTS AND DISCUSSION: Nuclear magnetic resonance analysis of the hydrogel components confirmed the modification of the HA with the adamantane, or cyclodextrin and methacrylate groups. An approximate 30% functionalization was achieved for each of the respective groups on the backbone of the HA. RGD and IKVAV conjugation was confirmed with the BCA assay as peptide remained on the HA backbone after extensive dialysis of the samples. Mixing of the two adamantane-HA and cyclodextrin-HA components in aqueous solution resulted in the formation of a hydrogel whose rheological properties could be characterized. Modulating the weight percentage of the hydrogel from 5% to 7.5% caused a shift in the mechanical properties of the hydrogel (Figure 1), with higher weight percentage hydrogels exhibiting increased relaxation times (0.999 seconds vs 2.512 seconds) and overall stiffness values (~1,100 pascals vs ~4,000 pascals). For future in vivo studies, a hydrogel formulation near 5 weight percent HA will be selected as this formulation results in mechanical properties similar to endogenous neural tissue. Importantly, altering the hydrogel weight percentage did not impact the shear- thinning or self-healing capabilities of the material. Hydrogel weight percentage did not impact the toxicity of the material towards primary mouse astrocytes after 24 hours of indirect exposure via Transwell. Altogether, the provided data indicates potential utility as a tissue engineering scaffold and bioactive agent delivery vehicle in the treatment of TBI.

Oximetry Probe Composite Hydrogels for Magnetic Resonance Imaging Detection of Oxygen Gradients In Vivo

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Cellular therapy is a promising tool for the treatment of complex pathologies like Type 1 Diabetes (T1D), cancer, and cardiovascular disease. Many cellular therapies target pathologies and regenerate tissue using cell-laden hydrogel devices. These cell-laden hydrogel devices, or macroencapsulation devices, require optimal oxygen transport to support cell viability and function. For example, islets harvested from donors or derived from stem cells may be used therapeutically to reverse T1D. However, islets and their cellular alternatives possess a higher oxygen consumption rate than other tissues to produce insulin and functionally cure T1D. Due to the oxygen demands of cells like islets, there is a need to measure oxygen within macroencapsulation devices in vivo to design an optimal macroencapsulation device for the success of cellular therapeutics.

Using various biomaterial approaches, we have engineered siloxane-based oximetry probe-laden hydrogel macroencapsulation devices to measure spatial oxygen distribution within macroencapsulation devices in vivo. We incorporated two different siloxane-based magnetic resonance (MR) oximetry probes – L3 and L6 with 2% and 5% surfactant – into alginate, agarose, and poly (ethylene glycol) (PEG) hydrogels. We performed testing of fluorescently labeled siloxane nanoprobe entrapment and retention in 1.5% alginate, 2% agarose, and 5% PEG hydrogels; hydrogels demonstrated variable retention in MR oximetry probe emulsions due to variation in hydrogel matrix structure and pore size. We evaluated the cytotoxic effects of hydrogel-entrapped MR oximetry probe on insulin-producing single beta cells in 2% agarose, 5% PEG, and 1.5% alginate with 0%, 5%, 10%, and 20% MR oximetry probe concentrations. We found that L6 nanoprobe with 2% surfactant exhibited the least amount of loss in cell viability for both encapsulated and free cells while L3 nanoprobe exhibited the highest amount of cell death. To mitigate the cytotoxic effects of the L3 nanoprobe, we fabricated polydimethylsiloxane (PDMS) microbeads using an emulsion technique and encapsulated L3 nanoprobe in the PDMS microbeads and found that this technique increased the function and viability of encapsulated beta cells. Finally, we performed MR oximetry measurements using magnetic resonance imaging (MRI), and we found that the nanoprobe-laden hydrogels as well as the L3 microbead hydrogels were able to successfully measure oxygenation within the macroencapsulation devices.

We have successfully developed an oximetry probe composite hydrogel that enables measurement of oxygen in cell-laden devices in vitro and in vivo. The design and development of this oximetry probe composite hydrogel will enable macroencapsulation device optimization for oxygen availability and ultimately improved function of encapsulated cell grafts.