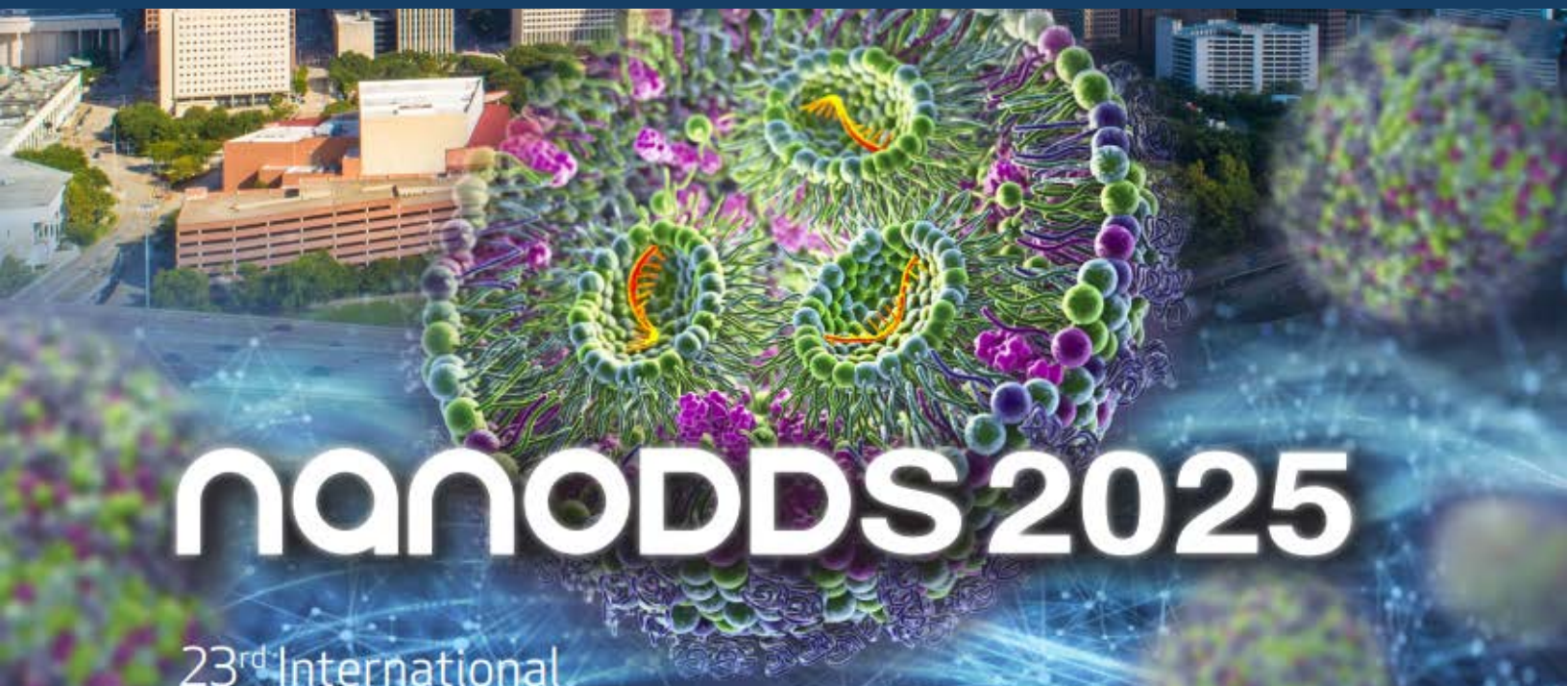
ABSTRACTS



nanoDDS2025

23rd International Nanomedicine and Drug Delivery Symposium

Co-sponsored by The George and Angelina Kostas Research Center for Cardiovascular Nanomedicine

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SPEAKER ABSTRACTS

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MONDAY, OCT 27

9:10am-9:50am	**Plenary** *A Hitchhiker's and Backpacker's Guide to Drug Delivery*
	***Samir Mitragotri*, PhD** *Hiller Professor of Bioengineering and Hansjörg Wyss Professor of Biologically Inspired Engineering, Harvard John A. Paulson School of Engineering and Applied Sciences, Wyss Institute at Harvard, Harvard University*
Abstract	Targeted delivery of drugs offers a potential to maximize their efficacy and minimize toxicity. To achieve this, drugs such as chemotherapeutics, proteins and mRNA are encapsulated in polymer or lipid-based carriers. However, upon injection in the body, these carriers face the challenge of poor blood circulation, limited targeting and the inability to negotiate many biological barriers. Biology has provided many examples of successful “carriers” in the form of circulatory cells including red blood cells, macrophages, neutrophils and T cells, among others, which routinely overcome the hurdles faced by synthetic carriers. We have developed “cellular hitchhiking and backpacking” approaches which involve combining synthetic carriers with circulatory cells to drastically alter the in vivo fate of the carriers as well as the cells. I will provide an overview of the principles and applications of hitchhiking and backpacking approaches for therapeutic delivery and cell therapy.
9:50am-10:55am	**Session 1: Nano-Immunotherapeutics**
9:50am-10:20am	***Keynote:*** *Supercharging Immunotherapy Through Nanotechnology: Chemical Structure Matters*
	***Natalie Artzi*, PhD** *Hansjörg Wyss Associate Professor of Biologically Inspired Engineering at Harvard Medical School, Head of Structural Nanomedicine at Mass General Brigham's Gene and Cell Therapy Institute, Associate Institute Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University*
Abstract	Structural nanomedicines are therapeutic constructs designed to maximize efficacy while minimizing toxicity by precisely organizing functional components. My lab designs biomaterials that overcome barriers to the spatiotemporal distribution of immunotherapies, enabling their combination with both existing and novel treatments. In this talk, I will demonstrate how material design and structure shape therapeutic outcomes, altering the spatiotemporal dynamics of immune responses in cancer treatment. Structural nanomedicines have the potential to answer fundamental questions in immunobiology and revolutionize treatments for cancer and autoimmune diseases

10:20am-10:40am	*Friends or Foes? Targeting Macrophages for Cancer Immunotherapy and Drug Delivery*
	***Wen Jiang*, MD, PhD** *Assistant Professor, Radiation Oncology, MD Anderson Cancer Center, Houston, Texas*
Abstract	Macrophages represent a central axis in cancer nanomedicine and immunotherapy: they can eliminate tumor cells through phagocytosis but also sequester therapeutic nanoparticles in the liver, limiting delivery. Here, I will present engineering strategies to reprogram macrophage activity to selectively enhance antitumor immunity while reducing off-target clearance. I will begin with our development of MUSIC (Nature Nanotechnology 2022), an ultrasound-guided platform that delivers the STING agonist cGAMP into antigen-presenting cells with spatiotemporal precision to amplify innate immune activation. I will then discuss a microbial-inspired CD47-listeriolysin O (LLO) fusion (Nature Cancer 2025) that disrupts the "don't eat me" checkpoint to drive robust tumor cell phagocytosis. Finally, I will highlight our recent work on MARCO blockade (Nature Nanotechnology 2023), which reduces hepatic macrophage uptake of nanoparticles and thereby enhances tumor delivery and therapeutic efficacy. Together, these efforts illustrate a convergent design strategy to reshape macrophage biology, transforming them from foes into allies for cancer nanomedicine and immunotherapy.
10:40am-10:55am	*Intratumoral delivery of a synergistic five-drug immunotherapy cocktail via a biodegradable nanoplatform eradicates cancer*
	***Corrine Ying Xuan Chua*, PhD** *Assistant Professor of Nanomedicine, Houston Methodist Academic Institute, Houston, TX*
Abstract	**Authors:** Jingyi Wang[1], Francesco Manfredi[1,2], Eleonora Molinari[1,2], Robin Vander Pol[1], Casey Lewis[1], Sally Peng[1], Alessandro Grattoni[1,3,4,], and **Corrine Ying Xuan Chua[1]** [1]Department of Nanomedicine, Houston Methodist Research Institute, [2]Department of Mechanical And Aerospace Engineering, Politecnico di Torino, [3]Department of Surgery, Houston Methodist Hospital, [5]Department of Radiation Oncology, Houston Methodist Hospital **Background.** The clinical potential of potent, multi-agent immunotherapies is unrealized due to severe systemic toxicities, a critical barrier to curative treatment. New delivery strategies are urgently needed to localize therapy, enabling unprecedented efficacy while eliminating systemic toxicity. To this end, intratumoral delivery can enhance efficacy and minimize toxicities by augmenting in situ bioavailability and limiting systemic drug exposure. However, clinically intratumoral delivery is affected by inconsistent injection techniques and rapid tumor leakage. **Methods.** We developed a biodegradable intratumoral drug-eluting seed for sustained localized immunotherapy delivery. The intratumoral seed is composed of biodegradable polymers, poly(ε-caprolactone)

	(PCL) and poly (D, L-lactic-co-glycolic acid) (PLGA) electrospun into a hollow cylindrical implant structure. Comparable in size to a grain of rice, the hollow central core acts as the drug reservoir. The intratumoral seed is minimally-invasively inserted in a one-time clinical trocar procedure. Controlled release occurs autonomously through the nanopores, enabling drug diffusion directly into the tumor. **Results.** Sustained intratumoral delivery of a synergistic 5-drug immunotherapeutic combination via the implant eradicated murine 4T1 triple negative breast cancer and KPC pancreatic cancer without triggering systemic toxicity. In contrast, conventional systemic delivery of the 5-drug combination resulted in cytokine storm and fatality. Further, intratumoral treatment enabled tumor immune microenvironment modulation into a "hot" phenotype. 4T1 mice with complete tumor clearance were metastasis free and had durable antitumor immunity as demonstrated by tumor rejection against rechallenge. In a bilateral KPC tumor model, abscopal responses to the 5-drug combination was achieved in contralateral untreated lesions. These data highlight the potency of the intratumoral seed for both local cancer treatment and systemic antitumor immunity. **Conclusion.** Overall, we demonstrate the efficacy and safety of an intratumorally delivered multi-drug immunotherapeutic cocktail, which is not feasible through conventional systemic administration due to toxicities. Our biodegradable sustained intratumoral delivery platform offers a safe and effective strategy for neoadjuvant immunotherapy or long-term treatment of inoperable tumors.
11:10am-12:35pm	**Session 2: Bio-Inspired Drug Delivery Systems**
11:10am-11:40am	***Keynote:*** *Engineering Cellular Nanoparticles for Multivalent Antigen Delivery*
	Liangfang Zhang, Ph.D.; *Joan and Irwin Jacobs, Chancellor Professor and Chair of the Aiiso Yufeng Li Family Department of Chemical and Nano Engineering at the University of California San Diego, San Diego, CA*
Abstract	Developing multivalent vaccines that target multiple antigens is critical for broadening and sustaining immune protection. By simultaneously stimulating immune responses against diverse epitopes from the same pathogen, or even across different pathogens, multivalent formulations increase the likelihood of generating robust and durable immunity while reducing the risk of vaccine failure due to antigenic drift, mutation, or pathogen heterogeneity. Building on our cell membrane coating technology, we have designed a broad-spectrum prophylactic cancer nanovaccine composed of induced pluripotent stem cell (iPSC) membranes wrapped around an adjuvant-loaded nanoparticle core. This nanostructure presents iPSC-derived oncofetal antigens, which are frequently re-expressed in cancer cells but minimally present in normal adult tissues. In parallel, by coating nanoparticles with bacterial membranes, we have developed multivalent nanovaccines capable of

	eliciting potent protection against a wide range of antimicrobial-resistant bacterial infections. In this talk, I will discuss our recent advances in multivalent nanovaccine platforms and their interactions with the immune system.
11:40am-12:10pm	*Bioengineering cell-based therapeutics from bench to bedside*
	Omid Veiseh, PhD *Professor of Bioengineering, CPRIT Scholar in Cancer Research* *Director of Rice Biotechnology Launch Pad, Rice University, Houston, TX*
Abstract	Cell-based therapeutics are an emerging modality that can potentially treat many currently intractable diseases through uniquely powerful modes of action. Our group is innovating new biomaterials and cellular constructs for medicine and biology by combining chemical biology, cellular engineering, and multi-scale fabrication. We have pioneered innovative approaches to synthesizing and in vivo screening of large libraries of biomaterial formulations for tailored applications in immunology and medicine. In my talk, I will describe our advances in discovering immunomodulatory biomaterials that can interact appropriately with the host immune system for localized immunomodulation. I will highlight our efforts to develop “cytokine factories” locally, activating both the innate and adaptive immune responses to generate systemic immunotherapy and eradicate metastatic cancer. This approach has advanced to phase I/II human clinical trials for treating recurrent, refractory ovarian cancer.
12:10pm-12:25pm	*Nanotechnology for Therapeutic Biocatalysis*
	Devleena Samanta, PhD *Assistant Professor, William H. Tonn Endowed Professorial Fellow,* *Department of Chemistry, UT Austin*
Abstract	Enzymes catalyze the vast majority of chemical reactions essential for life, making them central targets for therapeutic intervention. The ability to sense and precisely regulate enzymatic activity offers transformative opportunities for early disease detection and the development of advanced biocatalyst-based therapies. Nanotechnology provides a powerful platform to achieve this goal by enabling programmable strategies for monitoring and modulating biocatalysis at the molecular level. Our laboratory develops multifunctional nanostructures with three complementary capabilities. First, we design multifunctional nanostructures that detect proteases, a class of enzymes frequently dysregulated in cancer, neurodegeneration, and infectious disease, to enable minimally invasive diagnostic platforms. Second, we create nanostructures that bind disease-relevant enzymes and provide programmable on/off control in response to arbitrary, user-defined chemical cues. This establishes a foundation for enzyme therapeutics that are activated only under desired physiological or environmental stimuli. Third, we engineer nanomaterials that enhance enzyme activity by more than 20-fold without genetic or chemical modification. When combined with enzymes, these nanomaterials generate therapeutic systems that outperform native enzymes in killing multidrug-resistant bacteria and inhibiting biofilm formation..

	Together, our work establishes a unified framework for therapeutic biocatalysis powered by designer nanomaterials. These advances open new strategies for precision diagnostics and personalized medicine. **Acknowledgements** This work is supported by UT startup funds, the Packard Foundation, the Welch Foundation, an American Cancer Society Institutional Research Grant, and NSF CHE. **References** 1. Single-Molecule DNA Tweezers Enable Programmable Control of Enzyme Activity via Arbitrary Molecular Cues, *Angew. Chem.* **2025** (in press) 2. Discovery of Surface-Engineered Nanoparticles That Boost Enzyme Activity via High-Throughput Screening and Machine Learning, *Small* **2025**, e07126. 3. Conformationally Locked Peptide-DNA Nanostructures for CRISPR-Amplified Activity-Based Sensing, *Angew. Chem.* **2025**, *64*, e202500649. 4. Surface-Engineered Nanoparticles Enhance the Peroxidase Activity of Heme-Containing Proteins, *ACS Nano*, **2025**, *19*, 7117–7128. 5. DNA-Barcoded Plasmonic Nanostructures for Activity-Based Protease Sensing, *Angew. Chem.* **2024**, *63*, e202310964.
12:25pm-12:40pm	*Modulating the Prenatal Environment with Extracellular Vesicles: A Promising Strategy for Congenital Malformations*
	Bruna Coradetti, PhD *Assistant Professor, Center for Precision Environmental Health* *Baylor College of Medicine*
Abstract	Congenital malformations affect nearly 2 per 1,000 live births worldwide, with neural tube defects (NTDs) among the most devastating. NTDs, including anencephaly, craniorachischisis, and spina bifida (SB), result from incomplete neural tube closure during early development. The “two-hit” hypothesis of SB describes an initial closure failure exacerbated by ongoing exposure of the lesion to a hostile intrauterine environment. Current in utero surgical approaches can mitigate severity but remain highly invasive and risky for both mother and fetus, underscoring the need for less invasive, mechanism-based interventions. Leveraging the supportive role of amniotic fluid (AF) in fetal development, we are investigating extracellular vesicles (EVs) derived from AF-mesenchymal stem cells (AF-MSCs) as reconfigurable nanocarriers to establish a pro-regenerative intrauterine milieu. EVs naturally encapsulate regenerative and immunomodulatory cargo while overcoming the scalability and safety concerns of direct cell therapy. In biodistribution studies, EVs selectively accumulated in the uterus and yolk sac of pregnant mice, with no maternal toxicity or adverse effects on fetal viability, even in the drug-sensitive SWV-Fnn strain. In the clinically relevant Fkbp8 knockout SB model, continuous EV administration (10^9 particles/dose) during neurulation significantly reduced lesion size (from 5–8 mm to ~3 mm) and promoted vertebral closure at thoracic and lumbar levels. These findings establish AF-

	MSC–EVs as minimally invasive, precision nanomedicine candidates for prenatal therapy. By uniting nanoscale delivery, immune modulation, and regenerative repair, this strategy introduces a safer, adaptable approach to lessen the severity of congenital malformations before birth and redefine the therapeutic landscape of fetal medicine.
2:00pm-3:05pm	**Session 3: Nanomedicines for Organ Targeting**
2:00pm-2:30pm	***Keynote:*** *In vivo gene editing for treating hematopoietic disorders*
	Gang Bao, PhD* *Foyt Family Professor of Bioengineering; Professor of Chemistry and Materials Science & NanoEngineering, Rice University, Houston, TX*
Abstract	There are many hematopoietic single-gene disorders including sickle cell disease (SCD), beta-thalassemia and Wiskott-Aldrich Syndrome (WAS), affecting millions worldwide. Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) has been the only curative treatment; however, it is associated with significant risks and matched sibling donors are available for only a small percentage of patients. While great progresses have been made over the last decade in developing CRISPR/Cas based *ex vivo* gene editing therapy for single-gene disorders, including the FDA approval of Casgevy for curing SCD, significant challenges exist in applying *ex vivo* gene editing therapy, such as the high cost (~$2.2M per patient) and potential safety issues. In this talk I will discuss our efforts in treating SCD using gene editing approaches, including the analysis of unintended on-target large gene modifications, comparison of Cas orthologs for therapeutic editing at different loci in beta-globin, BCL11A and gamma-globin genes, and lipid nanoparticle based *in vivo* delivery for treating SCD. The challenges in achieving high *in vivo* editing efficiency will also be discussed.
2:30pm-2:50pm	*Kidney-Targeted Gene Therapy Delivery Via Polymeric Mesoscale Nanoparticles*
	Ryan Williams, PhD* Assistant Professor of Biomedical Engineering, City College of New York, NYC
Abstract	The last decade has seen a rapid expansion of the clinical utility of gene therapies. Many of these are focused on liver diseases, with a few exceptions. This is in large part due to the relative abundance of gene therapy carriers that target the liver and other accessible sites. However, there has been very limited success in the development of kidney-targeted gene therapy carriers to treat renal diseases. We are focused on the development of a non-viral gene delivery tool composed of FDA-approved polymeric materials to treat chronic kidney diseases. We optimized the formulation of kidney-targeted polymeric mesoscale nanoparticles (MNPs) to maximize the encapsulation of siRNA and mRNA gene therapies for kidney diseases. We developed a two-step

	mixture-nanoprecipitation formulation to design MNPs composed of poly-lactic-co-glycolic polyethylene glycol di-block (PLGA-PEG) polymer. Several MNP formulations loaded with reporter mRNA were evaluated for their ability to target the kidneys. Similarly, several MNP formulations loaded with siRNAs that target inflammatory cytokines (IL-6, IL-1β, TNFα) were evaluated in both mouse and rat models of chronic kidney disease. We assessed blood and urine markers of renal function in both models, as well as performed a histological assessment of renal inflammation. We optimized mesoscale nanoparticles for maximal siRNA and mRNA loading while maintaining a 300 – 400 nm polymeric MNP diameter for optimal kidney targeting. In healthy immunocompetent hairless mice, we found that MNPs specifically localize to the kidneys and deliver functional luciferase reporter mRNA. In both mouse and rat chronic kidney disease models (surgical and hypertensive disease models, respectively), we administered inflammatory cytokine-silencing siRNA-loaded MNPs. These studies demonstrated substantial therapeutic efficacy and reduction of inflammation from kidney-targeted siRNA delivery as measured by renal fibrosis and injury markers. MNPs localize to the kidneys with up to 26-fold specificity compared to other organs, therefore there is little to no off-target delivery or toxicity. We anticipate further pre-clinical development of both mRNA and siRNA-targeted delivery to the kidneys for chronic kidney diseases will result in a highly specific novel therapy with minimal off-target effects, a first-in-class indication for extrahepatic gene delivery.
2:50pm-3:05pm	*Nanotechnology-Driven Therapies for Neurodegenerative Diseases* ***Sonia Villapol, PhD*** *Assistant Professor of Neurosurgery, Houston Methodist Academic Institute, Center for Neuroregeneration, Department of Neurosurgery, Houston Methodist Research Institute, Houston, TX*
Abstract	**Introduction:** Neurological disorders, including Alzheimer's disease (AD), stroke, and traumatic brain injury (TBI), are leading causes of morbidity, disability, and mortality worldwide. A shared feature is blood-brain barrier (BBB) dysfunction, which compromises brain homeostasis and impedes effective therapy. AD pathology is marked by β-amyloid (Aβ) plaques, tau tangles, neuroinflammation, and neuronal loss, while TBI is a well-established risk factor for AD, accelerating neuroinflammation and pathological progression. Despite advances in rehabilitation, effective neuroprotective treatments for TBI patients remain lacking, and current drug delivery methods are inefficient in targeting inflamed brain regions. Nanomedicine offers a powerful strategy to overcome these barriers, enabling the selective delivery of therapeutics to injured or diseased brain tissue. **Hypothesis:** We have developed dexamethasone-loaded liposomal nanocarriers (Lipo-Dex) to suppress TBI-induced inflammation. Dexamethasone, a glucocorticoid receptor agonist, is widely used to reduce swelling and inflammation, but its systemic toxicity and poor BBB penetration limit its therapeutic use. Lipo-Dex is designed to selectively deliver dexamethasone to the injured brain, reducing neuroinflammation while minimizing systemic exposure. We hypothesize that delivery of Lipo-Dex, combined with siRNA-

	conjugated iron oxide nanoparticles (siRNA-IONPs) targeting JNK2 in microglia/macrophages, will suppress proinflammatory signaling, reduce neuroinflammation, and mitigate the progression of Aβ and tau pathology following TBI. **Methods:** *In vitro*, Lipo-Dex was well tolerated in human and murine neural cells and significantly suppressed lipopolysaccharide-induced IL-6 and TNF-α release. *In vivo*, young adult male and female C57BL/6 mice received Lipo-Dex immediately after controlled cortical impact injury, a mouse model of TBI. Therapeutic efficacy was also assessed in APP/PS1 and APP23 AD mouse models subjected to TBI, evaluating inflammation, Aβ and tau burden, and cognitive performance. siRNA-IONPs targeting JNK2 were tested alone and in combination with Lipo-Dex. Imaging (IVIS), molecular assays, RNAscope, and biochemical analyses were used to assess biodistribution, safety, and efficacy. **Results:** Lipo-Dex selectively accumulated in injured brain regions with reduced systemic exposure. In TBI mice, Lipo-Dex treatment decreased lesion volume, neuronal loss, astrogliosis, pro-inflammatory cytokine release, and microglial activation, with the strongest effects observed in males, highlighting sex as a critical variable in nanotherapy development. Toxicity profiling revealed no significant alterations in serum proteins or hepatic/renal markers (ALP, AST, ALT, BUN), confirming systemic safety. siRNA-IONPs effectively reduced JNK2 mRNA and protein expression in Iba1+ microglia, reprogramming them from a pro-inflammatory to a neuroprotective state. **Conclusions:** Lipo-Dex demonstrates strong potential as an acute TBI therapy by selectively targeting inflamed brain tissue and reducing secondary injury in a sex-dependent manner. In parallel, siRNA-IONPs targeting JNK2 provide a novel approach to reprogram microglia and mitigate chronic neuroinflammation that links TBI to AD progression. Together, these nanotechnology-driven strategies address critical limitations in BBB penetration and inflammatory modulation, offering a path toward effective, brain-targeted interventions for TBI and AD. **Acknowledgements:** This work was supported by grants from the National Institute of Neurological Disorders and Stroke (NINDS), R21 NS127265, and NIH grant R56AG080920 from the National Institute on Aging (NIA). **References:** 1. Zinger A, Soriano S, Baudo G, De Rosa E, Taraballi F, Villapol S. Biomimetic Nanoparticles as a theranostic tool for traumatic brain injury. Adv Funct Mater. 2021 Jul 23;31(30):2100722. PMID: 34413716. 2. Baudo G, Flinn H, Holcomb M, Tiwari A, Soriano S, Taraballi F, Godin B, Zinger A, Villapol S. Sex-dependent improvement in traumatic brain injury outcomes after liposomal delivery of dexamethasone in mice. Bioengineering & Translational Medicine. 2024; 9(4):e10647. doi:10.1002/btm2.10647

	3. Lopez-Espinosa J, Park P, Holcomb M, Godin B, Villapol S. Nanotechnology-driven therapies for neurodegenerative diseases: a comprehensive review. Ther Deliv. 2024;15(12):997-1024.
3:20pm-5:00pm	**Session 4: Embracing Nanomedicine Clinical Translation**
3:20pm-3:50pm	**Keynote:** *Promoting cancer nanomedicine clinical translation* ***Twan Lammers, PhD*** *Professor, Dept. of Nanomedicine and Theranostics, Institute for Experimental Molecular Imaging, RWTH Aachen University Clinic, Center for Biohybrid Medical Systems, Aachen, Germany*
Abstract	Nanomedicines are extensively used for cancer therapy. By delivering drug molecules more effectively and more selectively to pathological sites, nanomedicines assist in improving the balance between drug efficacy and toxicity. The tumor accumulation of nanomedicines is traditionally ascribed to the EPR effect, which is highly variable, both in animal models and in patients. To address issues associated with tumor targeting heterogeneity, and to advance cancer nanomedicine clinical translation, we are working on tools and technologies to predict, monitor and modulate tumor-targeted drug delivery. In the present lecture, several of these strategies will be highlighted, including ultrasound-based interventions to prime the tumor microenvironment, and the use of imaging and tissue biomarkers for patient stratification. Altogether, our work aims to establish rational and realistic ways forward towards improving the clinical performance of cancer nanomedicines.
3:50pm-4:00pm	*From Formulation to Clinic: Approaches to Scalable Nanomedicine Development* ***Silvie Meeuwissen, PhD*** *Director of Process Research & Development (PR&D) and Manufacturing Nanomedicines, Ardena*
Abstract	Translating nanomedicines from lab bench to clinical use is rarely straightforward. Upscaling a promising formulation requires early and deliberate attention to manufacturability and characterization. This presentation highlights how applying Quality by Design principles during development supports the creation of robust processes and analytical methods to successfully manufacture nanomedicines. Drawing on Ardena's experience as a specialist CDMO working with lipid-, polymer-, and metal-based nanoparticles, this presentation will explore common CMC challenges and practical solutions. Case studies will illustrate how to overcome development hurdles and advance nanomedicines safely and effectively toward clinical application.

**** Sponsored by George and Angelina Kostas Center for Cardiovascular Nanomedicine***

TUESDAY, OCT 28

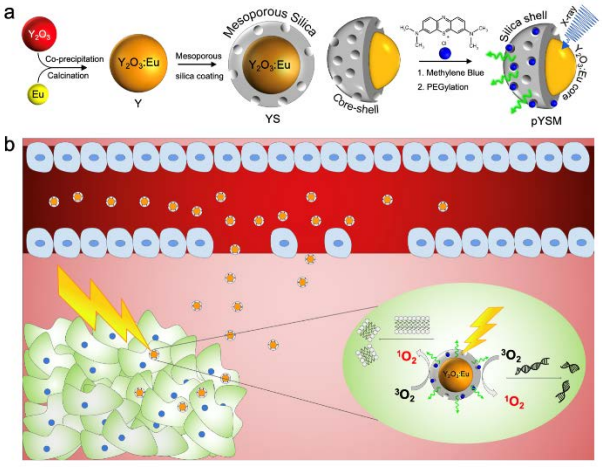
9:00am-9:40am	**Plenary** *Polyoxazolines for micelles and beyond* ***Aleksander (Sasha) Kabanov, PhD*** *Mescal Swain Ferguson Distinguished Professor* *Director, Center for Nanotechnology in Drug Delivery at the UNC Eshelman School of Pharmacy and co-director of the Carolina Institute for Nanomedicine at the University of North Carolina at Chapel Hill.*
Abstract	**Authors: Alexander Kabanov*,** Jacob Ramsey, Kyongtea Kim, Alyssa Holden, Hallie Hutsell, Liubov Palchak, Marina Sokolsky Poly(2-oxazoline) (POx)-based block copolymers form polymeric micelles (PMs) that enable exceptionally high loading of water-insoluble drugs, significantly enhancing their solubility, stability, efficacy, and safety. At drug loadings approaching ~50% by weight, the solubilized drug becomes a major structural component of the micelle, influencing key properties such as micelle stability, critical micelle concentration (CMC) and morphology. Certain drugs, including paclitaxel, interact favorably with POx block copolymers, stabilizing micelles by lowering the CMC and preserving spherical morphology—preventing elongation into worm-like structures. These drugs, termed **Group B**, also facilitate the co-loading and retention of **Group A** drugs, which otherwise incorporate poorly into the same polymer system. Micelle morphology directly impacts therapeutic performance: spherical micelles accumulate rapidly in tumors and demonstrate superior antitumor efficacy compared to worm-like micelles, which release drug more slowly and accumulate less efficiently. Moreover, super-high drug loading—translates into a greater amount of drug delivered to the tumor site, resulting in enhanced antitumor effects at the same drug overall dose. This dual advantage of optimized morphology and high payload underscores the potential of POx-based micelles as powerful platforms for cancer therapy. Key physical parameters—including drug loading, micelle-to-serum partition constant (drug retention), and CMC (micelle stability)—govern pharmacokinetics (PK), tumor distribution, and therapeutic efficacy. These parameters support PK/PD modeling and the development of a **mechanistic framework for in vitro–in vivo correlation (IVIVC) in PMs**, using in vitro measurable or computationally predicted equilibrium properties of drug–micelle interactions. To support clinical translation, scalable manufacturing approaches such as lyophilization and spray drying have been developed. Challenges related to storage, stability, and reconstitution of drug-loaded PMs from amorphous solid dispersions are addressed. This rational micelle design approach enhances therapeutic outcomes, streamlines formulation development, reduces animal use, and accelerates clinical translation.

	We and others have significantly expanded the applications of POx block copolymers beyond PMs, including their use in polyion complexes and POx–lipid conjugates to form lipid nanoparticles (LNPs) for nucleic acid delivery, POx-stabilized drug nanocrystal suspensions, and oral delivery systems that achieve unprecedented bioavailability of poorly soluble drugs. In these and other formulations, POx polymers consistently outperform PEG and other synthetic polymers due to their exceptional chemical diversity, stability, and robustness in manufacturing. Importantly, the lack of pre-existing anti-POx antibodies—in contrast to the well-documented presence of anti-PEG antibodies—offers hope that POx polymers may also provide a favorable safety profile, further supporting their penetration into diverse therapeutic applications. These advances strongly support the expectation that POx polymers will emerge as versatile new polymeric excipients in the healthcare sector, enabling next-generation drug delivery systems across a wide range of modalities. Acknowledgements: Funding from NIH CA198999, CA264488, CA305031. COI: DelAQUA Pharmaceuticals.
9:40am-10:55am	**Session 5: Advances in Polymeric Nanocarriers for Drug Delivery**
9:40am-10:05am	***Keynote:*** *Vascular-confined particles for thrombolysis*
	Paolo Decuzzi, PhD* *Senior Scientist and the Founding Director of the Laboratory of Nanotechnology for Precision Medicine at the Italian Institute of Technology in Genova – Italy.*
Abstract	Tissue-type plasminogen activator (tPA) is the only approved thrombolytic for acute ischemic stroke, but its use is limited by a narrow therapeutic window, rapid systemic inactivation, and heightened risk when the blood–brain barrier (BBB) is compromised. To improve efficacy and safety, we coupled clinical-grade tPA to soft, discoidal polymeric nanoconstructs (tPA-DPN) designed to confine drug activity within the vascular walls. The porous DPN matrix shields tPA from rapid degradation, preserving >70% enzymatic activity after 3 h in serum. In murine mesenteric venules, intravenous tPA-DPN (2.5 mg/kg) recanalized ~90% of occluded vessels versus ~40% with an equal dose of free tPA; at ~1/10 of the clinical dose (1.0 mg/kg), tPA-DPN cleared ~70% of clots compared with ~16% for free tPA. Discoidal tPA-DPN also outperformed 200-nm spherical tPA-coated constructs. In a transient middle cerebral artery occlusion model, free tPA worsened injury (24h survival ~10%), increased lesion volume by >2-fold, and raised BBB permeability by ~50%. In contrast, tPA-DPN yielded ~75% 24h survival (similar to saline) without increasing lesion size, BBB permeability, activity score, or neurological severity score. Together, robust tPA conjugation, favorable DPN deformability and circulation,

	and vascular confinement enhance thrombolysis while mitigating neurovascular toxicity, supporting tPA-DPN as a promising approach to improve the potency and safety of thrombolysis beyond the conventional acute window.
10:05am-10:30am	*Engineering polymer prodrugs for infectious disease and immune therapies*
	Patrick Stayton, PhD *Bioengineering Distinguished Term Professor, Director, Molecular Engineering and Sciences Institute, University of Washington*
Abstract	TBD
10:30am-10:50am	*Polymersomes Enable Delivery of Biologics Across the Blood-Brain and Blood-Nerve Barriers*
	Jessica Larsen, PhD *Associate Professor, Dept of Chemical and Biomolecular Engineering, Clemson University, SC*
Abstract	According to the World Health Organization, neurologic disorders account for the largest global burden through both early mortality and loss of independent life due to disability. Although there is a clear need for treatments that can enter the brain, there are a very limited number of medications available on the market. A major contributor to this fact is the presence of the blood-brain barrier (BBB), which prevents passage of more than 98% of small molecule therapeutics from the blood into the brain tissue. The Larsen Lab works on polymeric biomaterials-based approaches to bypass and transport payloads through the BBB and the blood-nerve barrier. By understanding the pathophysiology of each disease, polymeric nanoparticles can be chemically tailored to respond specifically to disease-based stimuli and promote natural healing processes in the brain. In this talk, Professor Larsen will highlight some of her ongoing projects in this space, specifically focusing on the following: 1. enzyme-responsive polymersomes that can promote self-healing autophagic processes and enzyme restoration in neuropathic lysosomal storage disease model, GM1 gangliosidosis. 2. pH-responsive polymersomes as delivery vehicles for nerve-regenerative peptides isolated from the G3BP1 protein, demonstrated to promote neurite outgrowth in dorsal root ganglia cultures, with some neuronal specificity. 3. Intranasal delivery of therapeutics to treat brain-eating amoeba.
11:05am-12:20pm	**Session 6: Gene Therapies**
11:05am-11:35am	***Keynote:*** *Lipid nanoparticles for overcoming biological barriers to cardiovascular mRNA delivery*
	Michael Mitchell, PhD ***** *Associate Professor of Bioengineering, UPenn* *Director, Lipid Nanoparticle Synthesis Core, Penn Institute for RNA Innovation, University of Pennsylvania, Philadelphia, PA*

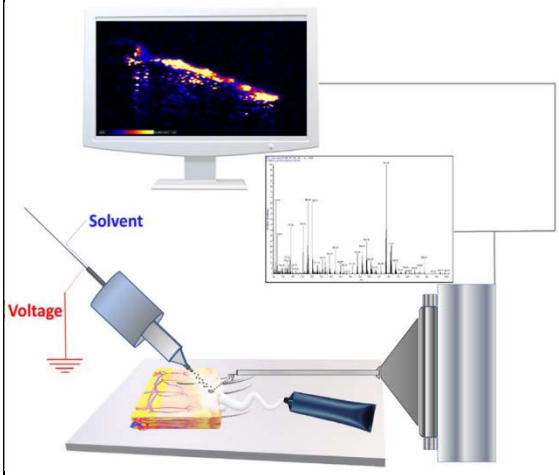
Abstract	Recent years have witnessed tremendous developments and breakthroughs in the field of RNA-based therapeutics and vaccines. The distinct mechanisms of exogenous RNAs and analogs, including messenger RNAs, small interfering RNAs, microRNAs, and antisense oligonucleotides, have brought them unprecedented potential to treat a variety of pathological conditions. However, the widespread application of RNA therapeutics and vaccines is hampered by their intrinsic features (e.g., instability, large size, and dense negative charge) and formidable host barriers. Development of safe and efficient vectors is key for successful delivery and translation of RNA therapeutics and vaccines. In this talk, I will discuss our efforts towards the development of new lipid nanoparticles (LNPs) that enable the delivery of RNA therapeutics and vaccines to target cells and tissues in vivo. Furthermore, I will describe new therapeutic strategies utilizing these LNPs for mRNA delivery to the heart to treat myocardial infarction.
11:35am-11:55pm	*Liver genome editing for cardiovascular disease*.
	William R. Lagor, PhD* *Kyle and Josephine Morrow Endowed Professor, Integrative Physiology, Baylor College of Medicine, Houston, TX*
Abstract	The liver plays a central role in lipid and lipoprotein metabolism, making it an attractive target for genome editing therapeutics. This organ is also readily targetable with Adeno-Associated Viral (AAV) vectors and lipid nanoparticles (LNP), which can efficiently deliver DNA templates and RNA respectively. The first generation of liver-directed genome editing therapeutics have focused primarily on disruption of genes either with CRISPR/Cas9 or base editors. Our lab has been working on editing the *LPA* gene as a way to lower lipoprotein(a) levels in the blood, the most commonly genetically determined risk factor for cardiovascular disease. *LPA* gene editing presents major challenges due to its repetitive structure and close homology to the fibrinolytic enzyme plasminogen (*PLG*). I will also discuss our efforts on improving the efficiency of precise gene correction in the liver through homology directed repair (HDR). We have developed a novel gene therapy platform that enables selective expansion of HDR-corrected hepatocytes, using transient inhibition of an essential gene. This technology, which we call “Repair Drive,” can increase the proportion of HDR-corrected hepatocytes from <1% to 25% in mouse liver. Repair Drive uses small interfering siRNA to eliminate unedited or incorrectly edited cells, providing space for precisely repaired hepatocytes to divide and regenerate the liver.
11:55pm-12:10pm	*Telomerase mRNA nanotherapy for protection from radiation-induced DNA damage in the skin*
	Anahita Mojiri, PhD* *Assistant Research Professor of Cardiovascular Sciences, Houston Methodist Academic Institute, Houston, TX*
	Shuang Li, David F. Chang, Karem A. Court, Thi Kim Cuc Nguyen, Vrutant V. Shah, Elisa Morales, Jack Carrier, Anjana Tiwari, Andrew T. Ludlow, Kristopher W. Brannan, Aldona J. Spiegel, Maham Rahimi, Jeffrey D. Friedman, Elizabeth Olmsted-Davis, Biana Godin, John P. Cooke, Anahita Mojiri.

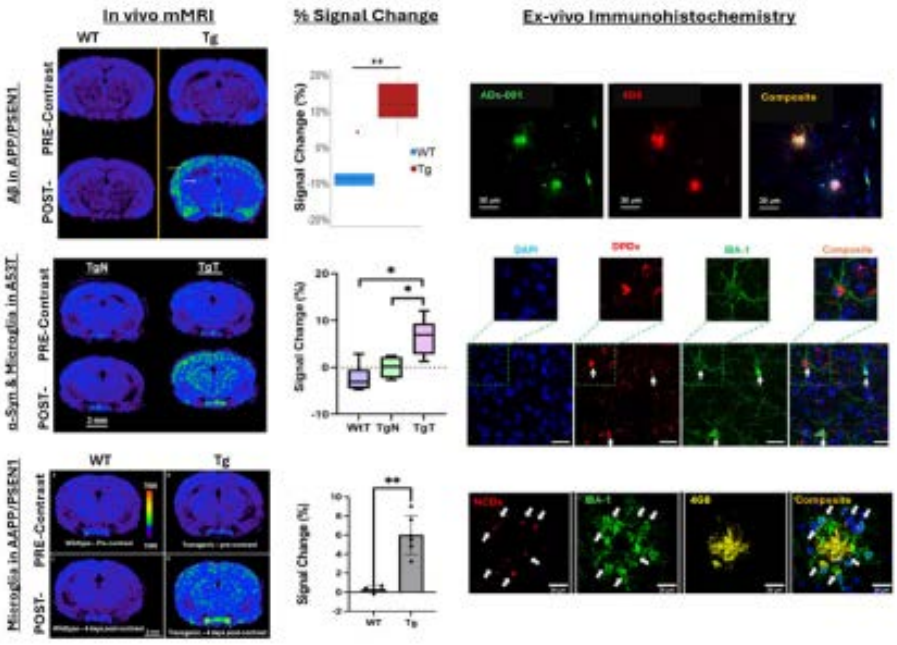
	Background. Radiotherapy is effective against tumors but frequently injures skin; there is no approved prophylaxis for radiation dermatitis. We asked whether lipid nanoparticle (LNP)-delivered telomerase (TERT) mRNA can provide transient, non-telomeric radioprotection by enhancing DNA repair and limiting mitochondrial stress. **Methods.** We evaluated TERT mRNA-LNP in primary human keratinocytes, fibroblasts, and microvascular endothelial cells, and in ex vivo human skin. Delivery was optimized by comparing formulations: a DOTAP-based LNP in cultured cells vs an ionizable MC3 LNP combined with microneedling for intradermal delivery in skin explants. Endpoints included gH2A.X (DNA double-strand breaks), TUNEL (apoptosis), mitochondrial ROS and membrane potential, AP sites (base damage), and p16/p21 (senescence markers). Telomerase activity and telomere length were assessed to distinguish non-telomeric mechanisms. **Results.** Ionizing radiation increased gH2A.X and mitochondrial injury across all skin cell types and induced apoptosis over time. Brief TERT mRNA-LNP pretreatment reduced gH2A.X, decreased early and late apoptosis, lowered mitochondrial ROS, and preserved membrane potential. Protection occurred without detectable telomere lengthening over the experimental window, supporting a non-telomeric repair function of TERT. In delivery optimization, DOTAP LNP outperformed standard transfection reagents in vitro, while MC3 LNP + microneedling achieved the highest transfection and measurable telomerase activity in ex vivo human skin. Skin pretreated with MC3-TERT mRNA LNP showed fewer AP sites, lower gH2A.X, and reduced p16/p21 induction after irradiation. **Conclusion.** LNP-mediated, transient TERT mRNA delivery, implemented with MC3 LNPs and microneedling, provides localized radioprotection to human skin via non-telomeric mechanisms. This platform emphasizes brief dosing, tissue-targeted delivery, and compatibility with clinical skin procedures, supporting its translational potential for preventing radiation induced dermatitis.
1:10pm-2:35pm	**Session 7: Imaging and Nanotheranostics**
1:10pm-1:40pm	**Keynote:** *Enabling light-activated therapies at depth using X-ray-triggered nanoscintillator luminescence*
	Sunil Krishnan, MD *Professor and John P. and Kathrine G. McGovern Distinguished ChairVivian L. Smith Department of Neurosurgery, McGovern Medical School at UTHealth Houston* *Professor, Center for Translational Cancer Research, The Brown Foundation Institute of Molecular Medicine at UTHealth Houston; Co-Director, Therapeutics and Pharmacology Graduate Program, MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences*

Abstract	Photoacoustic imaging, photothermal therapy, and photodynamic therapy are increasingly deployed for the diagnosis and treatment of superficial or endoscopically accessible tissues. However, the inability of light to traverse beyond 1 cm of tissue thickness remains a major challenge for the clinical deployment of these modalities in the diagnosis and treatment of tumors in deep-seated tissues. X-ray excited Photodynamic Therapy (XPDT) is a recently proposed modality for treating deep tissue tumors. In XPDT, a scintillating nanoparticle is conjugated to a photosensitizing compound, and under X-ray excitation, the nanoscintillator emits light which is then absorbed by the photosensitizer to generate reactive oxygen species (ROS). These ROS can then induce cell death via oxidative stress through DNA or lipid membrane damage (Figure 1). Due to the penetration depth and spatial localization of ionizing radiation systems, XPDT has the potential to supplement radiotherapy and decrease the radiation dose given to patients while improving the cytotoxic efficacy of radiotherapy. I will describe how we synthesized a nanoscintillator-photosensitizer complex containing Y_2O_3 doped with 5% Eu fluorescing at 611nm and decorated with SiO_2 containing the scintillation-coupled photosensitizer methylene blue and a polyethylene glycol coating (pYSM). When irradiated, pYSMs generate singlet oxygen species *in vitro*, causing cytotoxicity with hallmarks of immunogenic cell death (calreticulin translocation to the cell membrane). Intravenously administered pYSMs home passively to pancreatic tumor xenografts and, upon 10 Gy irradiation, cause significant tumor regression. On combining XPDT with anti-PD1 immunotherapy, a distant unirradiated tumor also regresses via an increase in intratumoral activated CD8+ cytotoxic T cells. Collectively, we advance a novel systemically delivered XPDT strategy that mediates an anti-tumor effect in both irradiated and unirradiated (abscopal) tumors when coupled with immunotherapy, converting a ‘cold’ ‘immune-desert’ tumor to a ‘hot’ immune-rich ‘inflamed’ tumor. 
1:40pm-2:05pm	*Nanoparticle-enhanced absorbable medical devices for vascular applications* ***Marites Pasuelo Melancon, PhD****

	Professor, Department of Interventional Radiology at The University of Texas, M.D. Anderson Cancer Center
Abstract	Cardiovascular diseases (CVDs) are the primary cause of mortality worldwide, driving a critical demand for advanced therapeutic devices and vascular replacements. Conventional solutions, such as permanent metallic implants (e.g., stents, filters) and non-resorbable synthetic grafts (e.g., ePTFE, PET), are limited by significant long-term complications including thrombosis, chronic inflammation, compliance mismatch, and infection, particularly in small-caliber applications. Our research involves the design and application of a new generation of bioresorbable, nanocomposite materials engineered to overcome these limitations through integrating nanotechnology and localized drug and cell delivery. First, we aim to improve the prevention of pulmonary embolism through the development of a fully absorbable inferior vena cava (IVC) filter. Fabricated from polydioxanone (PPDO), the device is rendered radiopaque for clinical imaging via the integration of metallic nanoparticles (e.g., AuNP, BiNP, WNP). Preclinical studies in a swine model demonstrate effective clot capture, predictable degradation kinetics, and complete absorption, thereby eliminating the need for costly retrieval procedures and mitigating the long-term risks associated with permanent metallic filters. Second, we aim to reduce the high failure rate of arteriovenous fistulas (AVFs) for hemodialysis access, which is primarily caused by neointimal hyperplasia. We have engineered an electrospun poly(ε-caprolactone) (PCL) perivascular wrap that provides mechanical support to stabilize hemodynamics at the anastomosis. This nanofibrous scaffold also serves as a local drug delivery system. In a rat model of chronic kidney disease, wraps loaded with drugs (e.g., rosuvastatin, rapamycin) or mesenchymal stem cells (MSCs) significantly attenuated inflammatory and proliferative pathways, leading to a marked reduction in neointimal hyperplasia and improved AVF maturation and patency. The inclusion of metallic nanoparticles further enables non-invasive CT tracking of the device in situ. Finally, we aim to create a novel bioresorbable vascular graft for small-diameter artery replacement. The electrospun graft is designed to facilitate in situ vascular regeneration, acting as a temporary scaffold for host cell infiltration and remodeling. The graft is embedded with metallic nanoparticles for real-time imaging of its position and resorption, and is loaded with anti-thrombotic agents to promote endothelialization and maintain long-term patency. In vivo studies in a rodent aortic replacement model confirm excellent biocompatibility and demonstrate cellular infiltration and the formation of a functional neovessel. Together, these platforms exemplify a theranostic approach, combining therapeutic delivery with diagnostic imaging and monitoring at the nanoscale. By engineering bio-interactive materials that dynamically support tissue regeneration before safely resorbing, we are paving the way for a new paradigm in the treatment of vascular disease, moving beyond inert implants to truly regenerative and curative solutions.

2:05pm-2:25pm	*Developing Mass Spectrometry Imaging-Guided Therapies and Drug Delivery Systems*
	Katy Margulis, PhD *Assistant Professor, The Faculty of Medicine-School of Pharmacy, The Institute for Drug Research, The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem, Israel*
Abstract	**Authors:** Ravit Yakobi-Arancibia, Einav Bentov-Arava, Reham Abu-Ghoush, **Katy Margulis** **Introduction:** We employ ambient mass spectrometry imaging, specifically Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI), to guide the rational design of drug delivery systems and to characterize endogenous molecular changes induced by drug administration. For dermal delivery, we developed a fast and sensitive method that integrates DESI-MSI with a novel automated computational tool, enabling precise targeting of active pharmaceutical ingredients (APIs) to distinct skin strata. Controlling drug localization across epidermal and dermal layers not only enhances treatment efficacy but also minimizes undesired systemic absorption. In parallel, we apply DESI-MSI to optimize nose-to-brain delivery of iron chelators in disorders associated with pathological brain iron accumulation. This approach allows direct monitoring of both drug distribution and neurotransmitter alterations induced by drug delivery. **Hypothesis:** We hypothesize that DESI-MSI can provide precise, spatially resolved insights to guide targeted delivery into specific skin strata and to enable the development of efficient nose-to-brain delivery systems. **Methods:** DESI-MSI work principle is quite simple (Fig. 1): a beam of charged solvent droplets is directed onto a thin tissue section to desorb and ionize molecules, while the splash of these droplets carries the resultant ions into a mass spectrometer for analysis. The tissue is moved at a controlled speed to record the mass spectra from different spatial coordinates, while the signals are subsequently converted into images of molecular ion distributions. **Results:** We established a rapid, label-free method for visualizing and comparatively quantifying API distribution across skin depths using DESI-MSI. To increase precision and reduce analysis time, we developed a novel automated spatial clustering tool that separates skin-specific signals from background, segments MSI data into thin, predefined layers starting from the stratum corneum, and improves quantitative accuracy. Validation against manual processing confirmed its reproducibility and reliability. We first applied this method to evaluate nanoscale terbinafine delivery systems designed to target distinct skin strata. Comparative studies in human and porcine skin revealed detailed permeation and localization patterns, while automated analysis accelerated kinetic assessments of skin penetration. This approach provided real-time feedback on delivery dynamics, enhancing the precision and safety of topical formulations. In parallel, we investigated intranasal delivery of deferiprone, an FDA-approved iron chelator, using an oil-in-water microemulsion. DESI-MSI confirmed its targeted brain distribution and limited peripheral

	exposure. Importantly, we demonstrated control over key neurotransmitters (dopamine, serotonin, glutamate) across multiple brain regions, showing therapeutic potential along with reduced systemic toxicity. **Conclusion(s):** Our results demonstrate that DESI-MSI is a powerful tool to guide the development of drug delivery systems. By enabling precise spatial mapping of both drug and endogenous molecular responses, DESI-MSI supports the rational design of more effective and safer dermal and intranasal therapies.  **Figure 1.** DESI-MSI work principle. A longitudinal section of human skin with a topical drug delivery system is depicted and the permeation depth of the drug is measured by DESI-MSI. **References:** Yakobi-Arancibia et al., A Rapid Assessment Approach for Skin Stratum-Targeted Drug Delivery Systems Using Mass Spectrometry Imaging and Spatial Clustering, *Small Science,* 2025, 5, 8.
2:25pm-2:35pm	*Precision Imaging of Neurodegenerative Disease Biomarkers: A Decade of Molecular MRI Nanoparticle Probe Innovation for Intracranial Disease Targets*
	Eric A. Tanifum, PhD. *Associate Professor, Edward B. Singleton Department of Radiology, Texas Children's Hospital; Department of Radiology, Baylor College of Medicine*
Abstract	The pathogeneses of neurodegenerative disorders are characterized by protein aggregation such as amyloid-β (Aβ) and hyperphosphorylated tau (tau) in Alzheimer's disease (AD) and alpha-synuclein(α-syn) in Parkinson's disease (PD), and neuroinflammation. Molecular imaging efforts to profile these biomarkers in vivo have been dominated by positron emission tomography (PET) imaging. Efforts to develop molecular magnetic resonance imaging (mMRI) have lagged but quietly gaining ground. Iron oxide nanoparticles and gadolinium Gd(III)-based agents have been engineered to target amyloid plaques, tau tangles, α-synuclein aggregates, and microglial activation. If fully realized, mMRI could offer transformative advantages over PET tracers: PET is limited by low resolution, ionizing radiation, high cost and limited accessibility. mMRI on the other hand can generate images with high resolution anatomy and functional details, is radiation-free, less expensive and more widely accessible. We propose to present a decade of targeted Gd(III)-based nanoparticle engineering for mMRI of biomarkers of neurodegenerative disorders in rodent models (**Figure 1**), including Aβ plaques (ADx)[1,2], oligomeric α-syn (DPDx)[3], and reactive microgliosis probes (NCDx). Our lead product, ADx-001 is

	currently in clinical trials as the first in humans mMRI probe for imaging Aβ plaques in AD patients.  **Figure 1.** Neurodegenerative disease biomarker targeted liposomes with an MRI contrast payload enable separation of transgenic mouse models of Alzheimer's and Parkinson's disease from controls via in vivo mMRI. In vivo data are validated by ex vivo immunohistochemical analyses. **References:** 1. Tanifum, E. A. et al. A Novel Liposomal Nanoparticle for the Imaging of Amyloid Plaque by Magnetic Resonance Imaging. J. Alzheimers Dis. JAD 52, 731–745 (2016). 2. Badachhape, A. A. et al. Pre-clinical magnetic resonance imaging of retroplacental clear space throughout gestation. Placenta 77, 1–7 (2019). 3. Sun, X. et al. A dual target molecular magnetic resonance imaging probe for noninvasive profiling of pathologic alpha-synuclein and microgliosis in a mouse model of Parkinson's disease. Front. Neurosci. 18, 1428736- (2024).
2:45pm-4:15pm	**Session 8: Organelles and Cell-Based Nanotherapies**
2:45pm-3:10pm	**Keynote:** *Cellular delivery of TRAIL to metastatic cells: A brief history and future outlook*
	Michael King, Ph.D. *E.D. Butcher Professor of Bioengineering, Department of Bioengineering, Rice University, Houston, TX*
Abstract	The remarkable discovery that fluid shear stress sensitizes a wide range of metastatic cancer cells to undergo TRAIL-mediated apoptosis has inspired several drug delivery approaches to target circulating tumor cells in the peripheral blood and lymphatic systems. In this talk I

	will summarize our work functionalizing circulating blood cells (leukocytes, platelets) with functional TRAIL protein using nanoscale liposomes, as well as the use of genetically engineered or synthetic blood cells for TRAIL delivery. We have tested these approaches in preclinical mouse models of metastatic cancer, as well as with primary blood samples collected from metastatic cancer patients. Current work focuses on tailoring liposomal formulations for specific oncogenic mutations, and achieving TRAIL sensitization noninvasively using focused ultrasound, defining a new approach we call “Cancer Mechanotherapy”.
3:10pm-3:30pm	*Enhancing synthetic nanoparticle tumor targeting by re-engineering with extracellular vesicles derived from cancer and immune cells*
	Santosh Aryal, PhD *Associate Professor, Pharmaceutical Science and Health Outcomes, UT Tyler, Tyler, TX*
Abstract	Intracellular communication maintains tissue and organ evolution across species, which is driven by cell-born nanoparticulate chaperones equipped to target cells and deliver the necessary information. These chaperones are cell-released membrane vesicles broadly defined as extracellular vesicles (EVs), which are differentiated based on their biogenesis, release pathways, size, content, and function. The content of EVs consists of lipids, nucleic acids, and, more specifically, proteins associated with the plasma membrane, organelles, and cytosol. Due to the presence of signature proteins from the plasma membrane and cytosol, EVs are well-equipped to recognize target cells; therefore, they are of great interest in targeted drug delivery technologies. However, clinical translation is challenging due to the innate heterogeneity caused by varied cellular environmental factors, limitations in isolation yield, and colloidal instability. Therefore, it is crucial to understand EV production and stability factors to ensure functional reproducibility. This talk will discuss the effect of environmental stress on EVs’ isolation, re-engineering with synthetic nanoparticles, and their tumor targeting. EVs were extracted from Natural Killer (NK) and cancer cells and re-engineered with a synthetic liposome and anticancer doxorubicin. This presentation concludes with the properties and findings of re-engineered EVs, such as therapy, safety, and biodistribution. **Acknowledgment:** National Institute of Biomedical Imaging and Bioengineering, 1R15EB036962-01.
3:30pm-3:50pm	*Extracellular vesicle-mediated mitochondria delivery for ischemic stroke therapy*
	Devika Soundara Manickam*, PhD *Associate Professor at Neurology, UTHealth, Houston, TX*
Abstract	Extracellular vesicles (EVs) are natural carriers of cellular cargo. EVs play a role in intercellular communication via transfer of their internal components, including lipids, proteins and nucleic acids. We study EV subsets that are either large or small: lEVs or sEVs isolated from human or mouse brain endothelial cells (BECs). Large EVs naturally incorporate

	mitochondria during their biogenesis. Our work has demonstrated that (1) lEVs but not sEVs contain mitochondria, (2) lEVs transfer mitochondria into recipient BECs and cortical and hippocampal neurons in mouse brain slices, (3) lEVs increase cellular ATP levels and mitochondrial function in the recipient oxygen- glucose deprived (OGD) BECs. Interestingly, recipient OGD BECs treated with lEVs displayed superior mitochondrial function (oxygen consumption- and extracellular acidification rates) compared to BECs treated with control sEVs that lack mitochondria. Moreover, lEVs isolated from donor BECs with compromised mitochondrial function failed to increase ATP levels in the recipient BECs—suggesting that the increased bioenergetics in the lEV-treated cells is a function of innate lEV mitochondria. (4) BEC-derived lEVs transfer their innate mitochondria that subsequently localized with recipient cell mitochondria in primary human BECs, suggestive of mitochon-drial fusion and (5) mice injected with lEVs demonstrated a 50% reduction in infarct volume and improved neurological functions (scored as behavioral recovery) compared to vehicle-injected mice in a mouse middle cerebral artery occlusion model of ischemia/reperfusion injury (stroke). We, for the first time, have demonstrated the therapeutic efficacy of the larger, mitochondria- containing EVs, in a mouse model of transient ischemic stroke. Delivery of functional mitochondria is an effective approach to protect the post-ischemic blood-brain barrier—and therefore is a promising strategy to decrease long-term neurological dysfunction post-stroke. Leveraging on our works developing BEC-derived lEVs containing mitochondria, we have also developed neuron-derived EVs and have demonstrated that (1) neuron-derived lEVs but not sEVs contain mitochondria, (2) lEV-treated recipient heat-stressed neurons showed increased cell survival, and (3) intramuscularly-injected mitochondria-containing lEVs were delivered to spinal cord motor neurons in an EV dose-dependent manner in healthy mice. These findings suggest that lEV mitochondria may decrease motor neuron degeneration via increasing mitochondrial function and thereby decrease early muscle denervation in a mouse model of amyotrophic lateral sclerosis. Overall, our works highlight the broad therapeutic potential of innate lEV mitochondria to treat a variety of nervous system pathologies associated with mitochondrial dysfunction.
3:50pm-4:05pm	*Mitochondrial transfer and replenishment: biological nanoparticles for metabolic remodeling in disease*
	Elvin Blanco, PhD* *Assistant Professor of Nanomedicine, Department of Nanomedicine, Houston Methodist Academic Institute, Houston, TX*
Abstract	**Introduction:** Mitochondrial dysfunction is a hallmark of various diseases, ranging from cancer to cardiovascular and neurodegenerative diseases. As an example, classically activated macrophages that exhibit dysregulated bioenergetics and

	increased glycolysis, sustain a pro-inflammatory microenvironment that drives atherosclerotic plaque progression. Similarly, macrophage polarization to a pro-inflammatory phenotype is crucial in the progression of nonalcoholic fatty liver disease (NAFLD) and its more severe form, nonalcoholic steatohepatitis (NASH). Amyloid beta (Aβ) in neuronal mitochondria leads to increased reactive oxygen species (ROS) production, decreased ATP production, and imbalanced mitochondrial dynamics of fusion and fission processes, ultimately culminating in neurodegeneration and synaptic abnormalities in Alzheimer's disease (AD). Taken together, avenues aimed at mitochondrial function restoration hold tremendous therapeutic potential. Herein, we highlight our efforts in atherosclerosis and AD. **Hypothesis:** Our hypothesis is that mitochondrial replenishment results in metabolic reprogramming and restoration of mitochondrial function in cells driving disease initiation and progression. **Methods:** In studies involving atherosclerosis, mitochondria were isolated from mouse livers and administered to classically activated bone marrow (BM)-derived macrophages (BMDMs). The effect of mitochondrial transfer on macrophage glycolysis, glycolytic drivers, and pro-inflammatory phenotype was assessed. Isolated mitochondria were administered intravenously to ApoE$^{-/-}$ mice fed a high fat diet and plaque burden was subsequently examined. In an AD setting, Aβ-treated SH-SY5Y cells were transfected with nuclear respiratory factor 1 (NRF1), and effects on bioenergetic handling, oxidative stress, and mitochondrial dynamics were assessed, along with neurodegenerative processes. **Results:** In the atherosclerosis setting, mitochondrial transfer had profound effects on cell bioenergetics, resulting in increased oxygen consumption rate (OCR) and reduced glycolytic flux that coincided with a decreased inflammatory response. Upon IV delivery to ApoE$^{-/-}$ mice fed a high fat diet, mitochondria accumulated in aortic plaques and co-localized with macrophages. Importantly, a reduction in plaque burden was observed. In the AD setting, neuronal cells exposed to Aβ and transfected with NRF1 had enhanced ATP production and reduced mitochondrial ROS production and mitochondrial membrane depolarization. NRF1 transfection maintained mitochondrial quality control through normalization of fission and fusion processes essential for healthy mitochondrial dynamics. Importantly, NRF1 induction abrogated Aβ-induced neuronal death and neurite shortening. **Conclusion:** Our findings highlight that mitochondrial replenishment strategies can counteract mitochondrial dysfunction and cell processes associated with disease progression, opening several avenues for innovative therapeutic approaches aimed at safeguarding mitochondrial health.
4:05pm-4:15pm	*From Detection to Quantification: Measuring EV Markers and Cargo using Nano-Flow Cytometry*
	James Fay, PhD

	NanoFCM
Abstract	The detection and quantification of molecular markers and cargos associated with nanoparticles, including extracellular vesicles (EVs), remain major challenges in biomedical research and therapeutic development. Here, we demonstrate the application of nano-flow technology for the detection of EV markers and introduce novel methods enabling quantitative single-particle analyses. While detection of EV markers by nano-flow cytometry is well established and qualitative assessments of marker expression are routinely obtained[1], advancements in tools and analytical approaches now allow determination of absolute copy numbers[2]. More recently, we developed a bead-based calibration cocktail that enables fluorescence intensity to copy-number conversion, providing accurate quantification of surface and internal EV markers. In addition, we engineered column-based cleanup strategies to eliminate staining artifacts, yielding low-background baselines and facilitating detection of minimally labeled particles. Collectively, this toolkit enables quantitative, single-particle readouts of EV marker abundance while maintaining the throughput and practicality of nano-flow assays. By emphasizing calibration, cleanup, and standardized analysis, the work provides a generalizable path to reproducible, copy-number resolved measurements across nanoparticle research and development.

**** Sponsored by George and Angelina Kostas Center for Cardiovascular Nanomedicine***

POSTER ABSTRACTS

Poster session I (Mon, Oct 27, 2025)

Imaging, theranostics, biosensors:
Posters #: 1, 6, 14, 16, 28, 33, 35, 55, 61, 69, 77, 78, 95, 98, 112
Nano-immuno:
Posters #: 3, 5, 8, 25, 29, 36, 39, 64, 88, 93, 111
Polymeric and peptide/protein based nanoparticles:
Posters #: 51, 53, 54, 60, 89, 96, 97, 101, 105, 107
Lipid nanoparticles and liposomes:
Poster #: 2, 4, 18, 30, 86, 99, 103, 110
Cell derived nanosystems:
Posters #: 7, 11, 13, 57, 58, 66, 67, 70, 71
Machine learning, AI, Mathematical modeling:
Posters #: 26, 49, 74

Metal and silica-based nanoparticles:
Posters #: 104, 113

Poster session II (Tue, Oct 28, 2025)

Gene (RNA/DNA) delivery:
Posters #: 12, 15, 17, 19, 40, 52, 56, 59, 65, 87, 100, 102, 108, 109

Organ and system targeting:
Posters #: 72, 73, 90, 92, 94, 106

Nano-devices:
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Poster session I (Mon, Oct 27, 2025):

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Poster session II (Tue, Oct 28, 2025)

Posters #: 12, 15, 17, 19, 22-23, 27, 31, 32, 34, 37, 38, 40-48, 50, 52, 56, 59, 63, 65, 72, 73, 87, 90-92, 94, 100, 102, 106, 108, 109

#1

pH-Sensitive Polymersomes as Photoacoustic Nanosensors for Receptor Labeling and Internalization Monitoring

Claire E. Jones[1], Ananthakrishnan Soundaram Jeevarathinam[1], Paula Aguilera Rodriguez[1], Jongyeong Jeon[2], Mohammad Kawelah[2], Alexander Marras[2,] Keith Johnston[2], Konstantin Sokolov[1*], and Richard Bouchard[1*]

[1] Department of Imaging Physics, MD Anderson Cancer Center, Houston, Texas 77030, United States.
[2] McKetta Department of Chemical Engineering, The University of Texas at Austin, Austin, Texas 78712, United States.
[*] RRBouchard@mdanderson.org and ksokolov@mdanderson.org

Introduction:
Antibody–drug conjugates (ADCs) are emerging therapies targeting specific cell-surface receptors, including trophoblast cell surface antigen 2 (Trop-2) [1]. Their efficacy and toxicity depend on receptor targeting and internalization, yet patient selection and monitoring still rely on invasive biopsies/procedures that preclude comprehensive or longitudinal assessment. To address this gap, we developed a pH-sensitive polymersome (PMpH) encapsulating J-aggregated indocyanine green (ICG) for photoacoustic-ultrasound (PA-US) imaging. This nanosensor design provides stable delivery, strong PA signal (~890 nm), and a built-in mechanism to report on cellular internalization through pH-triggered deaggregation (pH<6-6.5) and a spectral blueshift to ~780 nm. PMpH was directionally conjugated to anti-Trop2 antibodies (PMpH-Trop2) to assess labeling and internalization of Trop-2+ cancer cells validated by in vitro assays [2,3].

Hypothesis:
We hypothesize that PMpH-Trop2 nanoprobes provide molecularly specific, noninvasive photoacoustic monitoring of Trop-2+ cancer cell labeling and internalization, enabling functional assessment of ADC delivery.

Methods:
MDA-MB-468 triple-negative breast cancer (TNBC) cells were first plated on a 12-well plate at $4x10^5$ cells per well and incubated for 12 hours. The media was replaced for a 3-hour incubation with (1) no PM_{pH} (2) untargeted PM_{pH}, and (3) PM_{pH}-aTrop2. Separately, a set of cells was exposed for 24 hours to (1) no PM_{pH}, (2) PM_{pH}-aTrop2 for 24h (no PITSTOP2), and (3) PM_{pH}-aTrop2 (incubated with PITSTOP2 for 30 minutes prior to exposure). After exposure, cells were harvested, washed, and analyzed by UV–Vis spectroscopy; labeled cells were embedded in 8% gelatin phantoms for PA-US imaging (Vevo LAZR; 21 MHz; 680–940 nm). Internalization and receptor recycling were further analyzed by incubating cells with PMpH-Trop2 (3 OD, 3 hr), harvesting at sequential time-points, and labeling with α-Trop2-PE for flow cytometry on a Fortessa X-20.

Results:
UV-Vis and PAI confirmed TNBC labeling after 3 hours of incubation. At 24 hr, spectral peaks shifted from ~895 to ~780 nm in the PITSTOP2 group, signifying polymersome breakdown and pH-driven ICG deaggregation, whereas untreated cells showed no shift. Flow cytometry validated internalization and receptor recycling: Trop-2 surface receptors were blocked 0–4 hr post-exposure but returned to baseline availability by 8 hr.

Conclusion:
PMpH-Trop2 functions as an effective PA molecular probe for Trop-2+ cell labeling and internalization—two key sensing features for ADC planning and monitoring. Ongoing work is investigating endocytic pathways underlying PA spectral dynamics to refine probe design and translational applications.

References:
[1] O'Meara, T et al. Antibody-Drug Conjugates in Breast Cancer: The Road Towards Biologically-Informed Selection and Sequencing. Curr Oncol Rep., 27:68-79 (2025).

[2] Kawelah, M. et al. Antibody-Conjugated Polymersomes with Encapsulated Indocyanine Green J-Aggregates and High Near-Infrared Absorption for Molecular Photoacoustic Cancer Imaging. ACS Applied Materials & Interfaces, 16 (5) 1944-8244 (2024).

[3] C. Wood et al. Clinically translatable quantitative molecular photoacoustic imaging with liposome-encapsulated ICG J-aggregates. Nat Commun 12, 5410 (2021).

#2

Mechanistic Insights into mRNA–LNP Interactions: Role of Ionizable Lipid Content in Regulating mRNA Intracellular Release and Translation

Hoang Quan Truong [1], Cao Thuy Giang Nguyen [1], Urmila Kafle [1,2], Fanfei Meng [1,2 *]

[1] Department of Biomedical and Nutritional Sciences, University of Massachusetts Lowell, 3 Solomont Way, Lowell, MA 01854, USA
[2] Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Building 2, 4349 Martin Luther King Blvd, Houston, TX 77204, USA

* Corresponding author:
Fanfei Meng, Ph.D.
Phone: 713-743-2629
E-mail: fmeng6@uh.edu

Keywords: mRNA, LNPs, Endosomal escape, Intracellular release, mRNA-LNP interaction, Dissociation

Introduction: Messenger RNA (mRNA) therapeutics have emerged as a transformative platform for the treatment of a wide range of diseases, including infectious diseases, cancer, and autoimmune disorders. Despite its promise, the clinical translation of mRNA faces significant challenges. Notably, mRNA is susceptible to degradation via oxidation, hydrolysis, and nucleases, and its large, negatively charged, hydrophilic structure hinders its ability to cross cell membranes, limiting intracellular delivery. Lipid nanoparticles (LNPs) have become the gold standard for nucleic acid delivery and are integral to the clinical success of mRNA-based therapeutics. LNPs protect fragile mRNA molecules, facilitate cellular uptake, and promote cytosolic delivery. A typical LNP formulation consists of ionizable lipids, cholesterol, phospholipids, and PEGylated lipids. Among these, ionizable lipids typically comprise the majority of the lipid content in LNPs, approximately 50 mol% in most clinically approved formulations. They play a pivotal role by enabling mRNA encapsulation during formulation, protecting mRNA from degradation in blood circulation, and promoting endosomal escape.

While endosomal escape is widely recognized as a key step for efficient mRNA delivery and expression, the subsequent release of mRNA from the LNP complex remains underexplored. Importantly, successful cytosolic localization does not guarantee translation; mRNA must first dissociate from the LNP to be accessible to the translational machinery. Because mRNA-LNP interactions are primarily governed by electrostatic forces between the mRNA and ionizable lipids, the amount of ionizable lipids may influence mRNA dissociation: higher ionizable lipid content may strengthen mRNA-lipid interactions, potentially impeding release, while lower content may facilitate dissociation.

Hypothesis: We hypothesize that adjusting the ionizable lipid content within LNPs can modulate mRNA dissociation, thereby optimizing transfection efficiency.

Method: To test this, we systematically varied the ionizable lipid content, specifically SM-102 and ALC-0315, in clinically approved LNP formulations from Moderna and Pfizer/BioNTech. We evaluated how changes in ionizable lipid content affect the physicochemical properties of LNPs (size, zeta potential, encapsulation efficiency), cellular uptake, endosomal escape, intracellular mRNA release, protein expression, and immunogenicity.

Results: Unexpectedly, LNPs formulated with a lower ionizable lipid content demonstrated significantly enhanced mRNA transfection efficiency and immunogenicity in murine models compared to the clinically approved mRNA/LNP formulations with higher ionizable lipid content (46.3–50 mol%). Notably, while endosomal escape was comparable across groups, formulations with reduced ionizable lipid content showed significantly greater intracellular mRNA release, suggesting that dissociation—rather than escape alone—may be a limiting factor in translation efficiency. These findings underscore the importance of striking a balance between mRNA–LNP interactions to facilitate effective mRNA release and functional translation.

Conclusion: Our results highlight a previously underappreciated aspect of mRNA delivery: the intracellular dissociation of mRNA from LNPs. This insight suggests that future optimization of mRNA-LNP formulations should consider not only cellular uptake and endosomal escape but also the intracellular release of mRNA as a key determinant of transfection efficiency.

#3

Immunogenic silica nanoparticles and silicified cells for ovarian cancer immunotherapy

L. Tang [a,b], B. Marwedel [c,d], C. Dang [a,e], M. Olewine [a,f], A. Noureddine [a], R. E. Serda [c]

[a] *Chemical and Biological Engineering, University of New Mexico, Albuquerque, NM, USA*
[b] *School of Medicine, University of New Mexico, Albuquerque, NM, USA*
[c] *Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA*
[d] *Biomedical Engineering, Cockrell School of Engineering, The University of Texas at Austin, Austin, TX, USA*
[e] *Bioengineering, Rice University, Houston, TX, USA*
[f] *Chemical Engineering, University of California Los Angeles, CA, USA*

Keywords: ovarian cancer, nanoparticles, cell-based therapy, immunotherapy

Introduction. Ovarian cancer immunotherapy remains a challenge due to the "cold" tumor microenvironment. One of the major limitations to the effectiveness of cancer immunotherapy is the influence of the tumor microenvironment, which is predominately immunosuppressive and hinders the ability of immune cells to function effectively. Combination therapy involving the co-delivery of multiple molecules promises to harness the advantages of several compounds spatially co-localized within a mother carrier. It is admitted that activation of the innate immune system can stimulate antigen presentation to the adaptive immune system by enhancing antigen-specific immune responses by Toll like receptor (TLR) signaling in APCs. The advantage of co-delivery of both TLR receptor agonists is multivalent activation of surface and endosomal TLRs on antigen presenting cells. In addition to enhancing endocytosis, TLR ligands enhance fusion with the MHC II, expression of co-stimulatory molecules and drive T-cell polarization via cytokine production. Combining TLR4 and TLR9 agonists has proven to amplify anti-tumor immune effect by polarizing macrophages, DC and natural killer cells towards a T-helper-(Th)1 phenotype

Hypothesis. We hypothesize that an immunogenic system co-presenting complementary bioactive molecules including the cancer-specific antigens (in case of cell-based tech), nucleic acid-based adjuvant Cytosine-p-linked to Guanine (CpG, TLR3/9 agonist), lipid-based adjuvant (MPLA, TLR4 agonist) on the surface will help turning the ovarian cancer microenvironment into "hot" aka, immune-responsive.

Methods and Results. Herein we present a rational design to create two distinct immunogenic systems: 1) cell-based and 2) nanoparticle-based technologies that both share their capacity to stand as a multi-agent platform that promotes immune response in a mouse model of ovarian cancer. The optimization of the hybrid nanosystem in terms of composition, charge is carried out using a matrix of characterization techniques including IVIS, immune assays for immune cells counting, confocal microscopy and flow cytometry to detect cell targeting within the tumor tissue and activation of antigen presenting cells. We highlight 1) increased survival of mice with advanced ovarian cancer when using CpG and MPL together, 2) Targeting of myeloid cells with particle-bound TLR agonists activates signaling pathways that upregulate expression of costimulatory molecules (e.g. CD40, CD80) and major histocompatibility complex (MHC), reestablishing immune activating antigen presentation and clonal expansion of antigen specific T cells and furthermore 3) intraperitoneal administration of silica-based nanosystems led to preferential accumulation within tumor-burdened tissues with selective accumulation in myeloid cells and superior efficacy compared to intravenous and subcutaneous injections.

Conclusion. The presented technology shows that intraperitoneal injection of immunogenic nanoplatform in advanced ovarian cancer targets myeloid cells, activates immune response, and highlights a promising platform for the treatment of peritoneal cancers[1].

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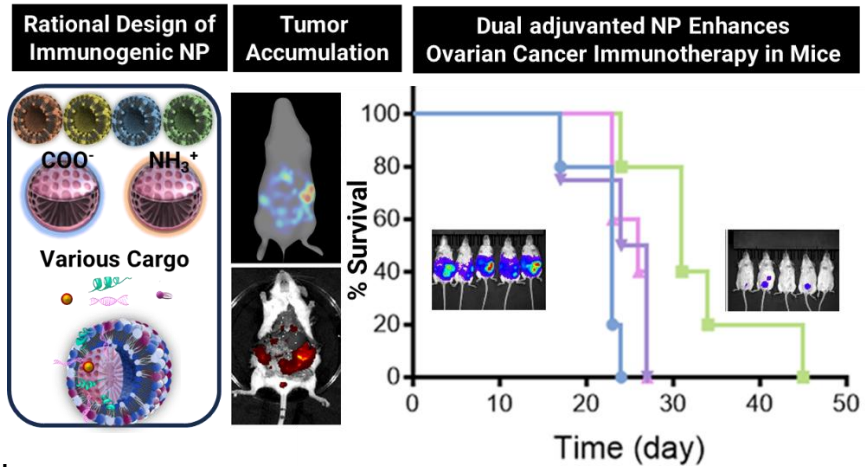

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#4

Optimized Liposomal Formulations of Rapamycin Improve Antitumor Efficacy in a Pre-Clinical Model of Epithelioid Hemangioendothelioma

Zilu Chen[1], Linyu Fan[1], Jasmine Lee[2], Albiruni R. A. Razak[2], Christine Allen[1, 3]

(1) Leslie Dan Faculty of Pharmacy, University of Toronto; (2) Princess Margaret Cancer Centre, University Health Network; (3) Department of Chemical Engineering & Applied Chemistry, University of Toronto

Keywords
liposomes; rapamycin; epithelioid hemangioendothelioma; nanoparticle drug delivery; rare cancers

Introduction

Epithelioid hemangioendothelioma (EHE) is a rare vascular sarcoma characterized by WWTR-CAMTA1 or YAP1-TFE3 gene fusions. Patients with progressive disease face poor outcomes, as no approved standard of care exists.[1] Rapamycin, a large hydrophobic mTOR inhibitor, has shown clinical activity in EHE but its current formulations are limited by low oral bioavailability, and off-target toxicity, necessitating novel delivery strategies to improve its efficacy while minimizing toxicity.[2] Liposomal nanoparticles offer the potential to improve pharmacokinetics, reduce systemic exposure, and achieve tumor-targeted delivery.[3] This study aimed to design, optimize, and evaluate liposome formulations of rapamycin with improved circulation time, controlled release, and superior anti-tumor activity in preclinical EHE models.

Hypothesis

We hypothesized that optimized liposomal rapamycin formulations would prolong circulation time, enhance tumor drug accumulation, and thereby improve antitumor efficacy compared to existing rapamycin formulations, while reducing off-target toxicity.

Methods

Two liposomal formulations were prepared via thin-film hydration using passive and active remote loading approaches. Various lipid compositions were investigated to optimize stability and encapsulation, including combinations of DSPC, DPPC, cholesterol, and DSPE-PEG2000. The particle size, polydispersity, and zeta potential of the formulation were evaluated. Encapsulation efficiency (EE%) was quantified by HPLC after separation of free and liposomal drug using size-exclusion chromatography. Drug release kinetics was assessed under physiologically relevant conditions (37 °C, 45 mg/ml BSA in PBS with 10% FBS) over 4 days. Stability was evaluated over time at 4 °C for 21 days. In vivo efficacy was studied in SCID mice bearing subcutaneous EHE xenografts, with tumor growth inhibition monitored over multiple treatment cycles for more than 3 months.

Results

Actively loaded liposomes achieved an encapsulation efficiency of 63 ± 5 %, uniform particle size (103 ± nm), and a negative surface charge (–22.7 mV), supporting colloidal stability. These liposomes demonstrated slower drug release than passively loaded liposomes, indicating improved intraliposomal retention. Passively loaded liposomes exhibited slightly higher encapsulation efficiency (73 ± 5 %) and superior long-term stability during refrigerated storage.

In vivo, both liposomal formulations demonstrated significantly greater tumor growth inhibition compared to nab-sirolimus, a recently approved albumin-bound nanoparticle formulation of rapamycin. Actively loaded liposomes produced the most pronounced effect, achieving > 40% tumor volume reduction with three weeks of treatment at 7.5 mg/kg and prolonged tumor suppression for > 8 weeks after treatment cessation.

Conclusions

We developed two liposomal formulations of rapamycin with favorable physico-chemical properties, prolonged release and improved antitumor activity in a preclinical EHE model. These results support liposomal rapamycin, particularly the actively loaded formulation, as a promising candidate for translational development. Further pharmacokinetic, biodistribution, and toxicity studies are warranted to enable clinical evaluation in patients with EHE, where treatment options are scarce.

Acknowledgements

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#5

Fight Osteoporosis with Osteoimmunomodulatory Engineered Delivery Strategies

Francesca Taraballi PhD [1,2], Federica Banche-Niclot PhD [1,2]

[1] Center for Musculoskeletal Regeneration, Houston Methodist Academic Institute, Houston, TX, USA
[2] Orthopedics and Sports Medicine, Houston Methodist Hospital, Houston, TX, USA

Keywords. Osteoporosis, ICOS-Fc, mesoporous silica particles, PLGA nanoparticles

Introduction. Osteoporosis is an excess-resorption bone disorder in which T-cell–driven inflammation elevates RANKL and osteoclast activity, disrupting the balance between bone formation and resorption. An emerging aspect of osteoimmunology centers on the interaction between inducible co-stimulator (ICOS) expressed on activated T-cells and its ligand ICOSL[1]. Beyond its canonical immunoregulatory role, recent evidence demonstrates that the ICOS/ICOSL pathway is also involved in bone turnover regulation[1], constituting an osteo-immune checkpoint directly relevant to osteoporosis. Notably, osteoclasts express ICOSL and its engagement by a recombinant soluble ICOS fusion protein (ICOS-Fc) triggers reverse signaling that suppresses osteoclast differentiation and function both *in vitro* and *in vivo*[2]. In such a contest, the study aims to engineer "smart" nanobiomaterials that combine chemical and biological cues to stimulate bone regeneration by delivering ICOS-Fc with tunable release profiles. Three complementary delivery strategies have been developed. First, ICOS-Fc was coupled to osteogenic inorganic particles (strontium-containing mesoporous bioactive glasses, Sr-MBGs) via two routes: surface grafting[2] and incorporation into Sr-MBG-based capsules. In parallel, ICOS-Fc was encapsulated within resorbable polymeric particles[3].

Hypothesis. Controlled ICOS-Fc release from nanocarriers engages ICOSL reverse signaling in osteoclasts, reducing osteoclastogenesis and thus helping restore bone remodeling homeostasis in osteoporosis.

Methods.
ICOS-Fc surface grafting on MBGs: Sr-MBGs were obtained via either conventional sol-gel synthesis or spray-drying process. The surface of Sr-MBG was functionalized with amine groups (Sr-MBG-NH_2) by reacting surface silanol groups. Subsequently, ICOS-Fc was immobilized on Sr-MBG-NH_2 via carbodiimide coupling with EDC/NHS activation of protein carboxylates, which enabled covalent linkage to surface amines. The functionalized inorganic particles were then incubated in ICOS-Fc solution for 2 hours at room temperature to complete the conjugation reaction.
ICOS-Fc incorporation in MBGs capsules: Sr-MBGs particles with an average size of 600 nm were used. The biomolecule was incorporated by co-spray drying a suspension of Sr-MBG particles with an ICOS-Fc solution using a BUCHI Mini Spray Dryer B-290 instrument. The spray-drying parameters and feed solutions were optimized to achieve well-defined microcapsules morphology.
PLGA particles containing ICOS-Fc: To enable controlled spatiotemporal delivery of ICOS-Fc, PLGA was selected for its for biocompatibility, regulatory acceptance, and finely tunable release profiles. Double solvent evaporation method was used to develop ICOS-Fc–loaded PLGA nanoparticles. Release profiles and biocompatibility using MC3T3-E1 cells line were assessed.

Results. Nitrogen physisorption analysis and FTIR confirmed efficient -NH_2 surface functionalization of Sr-MBGs while TGA verified successful ICOS-Fc grafting. Particles were biocompatible with MC3T3-E1, engaged ICOSL and inhibited osteoclasts migration and differentiation *in vitro*. The microcapsules were near-spherical (~30 μm) with a densely packed morphology. Biocompatible PLGA nanoparticles showed a narrow size distribution peaking at ~300 nm and ~33% loading efficiency. ICOS-Fc functionality–both anchored to MBGs surface and encapsulated into MBG-based and PLGA capsules–was assessed by ELISA-like assay.

Conclusions. Coupling ICOS-Fc to Sr-containing MBGs proved a promising strategy to synergize antiresorptive and pro-osteogenic effects, thereby accelerating bone repair. Further studies are required to optimize ICOS-Fc loading in Sr-MBG microcapsules and PLGA nanoparticles and tune their degradation to achieve the targeted therapeutic concentrations.

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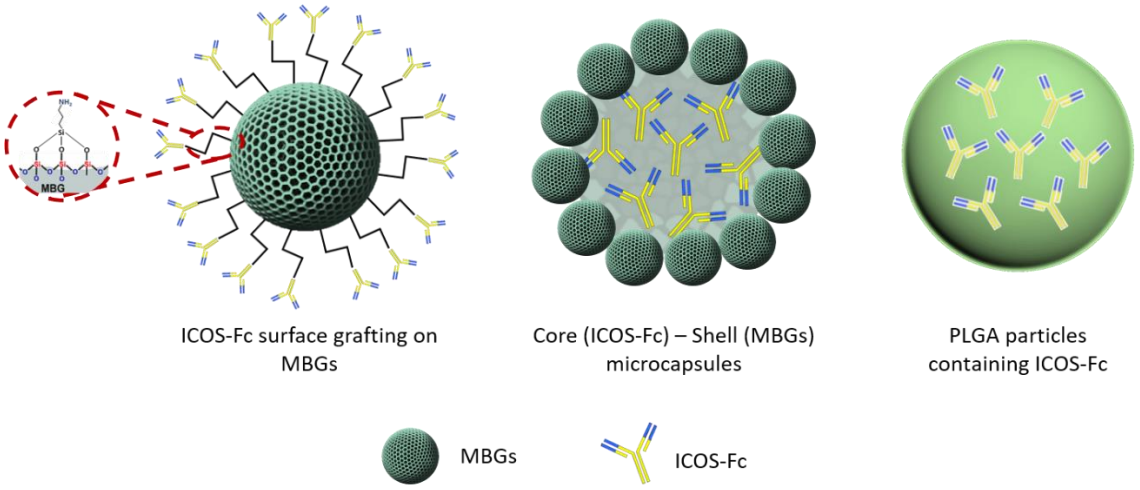

Figure 1. Schematic representation of the three different osteoimmunomodulatory carriers.

#6

Tile: Targeted, paclitaxel-carrying polymersomes with J-aggregates of indocyanine green in the aqueous core for photoacoustic image-guided chemotherapy.

Jeevarathinam AS [1], Kawelah MR [2], Dincer CA [2, 3], Han S [1,4], John S [1], Brisola J [2], Dileep J [2], Kamath AA[2], Chen M[2], Bouchard R[1], Marras AE [5,6], Truskett TM [5, 6], Johnston KP [2, 6], Sokolov KV [1, 4, 7]

[1] Department of Imaging Physics, MD Anderson Cancer Center, Houston, Texas; [2] McKetta Department of Chemical Engineering, The University of Texas, Austin (UTA), Texas; [3] Department of Chemical Engineering, Faculty of Engineering, Ankara University, Ankara, Turkey; [4] Department of Bioengineering, Rice University, Houston, Texas; [5] Walker Department of Mechanical Engineering, UTA; [6] Texas Materials Institute, UTA; [7] Graduate School of Biomedical Sciences, The University of Texas MD Anderson Cancer Center, Houston, Texas

Keywords: polymersome, drug delivery, , directional conjugation, monoclonal antibody, cancer therapy,

Introduction: In this study, we describe PLGA-*b*-PEG polymersomes containing J- aggregates of indocyanine green dye at the aqueous core and paclitaxel in the bilayer shell (PTX-ICGJ-Ps). The nanoparticles exhibit a strong absorbance at 895 nm along with 10% w/w loading of paclitaxel. PTX-ICGJ-Ps exhibit an excellent photoacoustic signal and spectral stability upon continuous exposure to 880 nm laser. We used anti-human epithelial growth factor receptor (aEGFR) antibody to specifically target triple negative breast cancer cells that express EGFR. The evaluation of EGFR-targeted PTX-ICGJ-PS (PTX-ICGJ-Ps-aEGFR) as an effective PAI trackable drug delivery agent is presented in this study.

Hypothesis: PTX-ICGJ-Ps-aEGFR wil enable efficient, molecular specific delivery of paclitaxel to EGFR-expressing cancer cells that will result in higher cytotoxicity to cancer cells as compared to either non-targeted, PTX-ICGJ-Ps, nanoparticles or the free drug. Furthermore, the delivery of PTX-ICGJ-Ps-aEGFR can be monitored by PAI.

Result: Flow cytometry and PAI showed highly specific targeting of EGFR-expressing cancer cells with minimum non-specific interactions. Since delivery of chemotherapeutic drug loaded nanoparticles via systemic administration is limited by the short blood circulation time, we evaluated PTX-ICGJ-Ps-aEGFR under conditions mimicking the rapid clearance of nanoparticles by incubating EGFR-expressing MDA-MB-468 cells with serial dilutions of PTX-ICGJ-Ps-aEGFR dispersed in full cell culture media for just 30 minutes. PTX-ICGJ-Ps-aEGFR exhibited significant cytotoxicity after 48 hours following the treatment. PTX-ICGJ-Ps nanoparticles and the free drug control showed a cytotoxic effect only after 72 hours and at higher drug concentrations as compared to the targeted PTX-ICGJ-Ps-aEGFR formulation.

Conclusions: We showed that PTX-ICGJ-Ps-aEGFR can be specifically targeted to triple negative breast cancer cells and are more efficient in delivery of a cytotoxic dose to cancer cells during a short exposure as compared to untargeted PTX-ICGJ-Ps and the free drug. Further, PTX-ICGJ-Ps-aEGFR can be detected using PAI with a high sensitivity.

Acknowledgements: This study was supported by the grants from Cancer Prevention & Research Institute of Texas (CPRIT) RP240311.

#7

Title: Development of brain endothelial cell-derived extracellular vesicles from bioreactor cultures

Govindaswamy B [1], Pinky PP [2], Basudkar V [2], Manickam DS [1]
[1] Department of Neurology, McGovern Medical School, UTHealth, Houston, TX; [2] Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA.

Keywords: Extracellular vesicles, bioreactor, brain endothelial cells, small extracellular vesicles, large extracellular vesicles, ATP restoration, bioenergetics

Introduction: Extracellular vesicles (**EVs**) are carriers of cellular cargo such as proteins, lipids, and nucleic acids that mediate intercellular communication and hold promise as a novel therapeutic/diagnostic platform. Efficient and scalable production of EVs remains a critical challenge for translational applications. Our laboratory has previously established a batch isolation process for EVs from cultured brain endothelial cells (**BECs**) grown in T-175 tissue culture flasks.[1–3]

Hypothesis: We *hypothesize* that bioreactor cultures of brain endothelial cells will support continuous collection of EVs resulting in high EV yields with preserved biological functionality and will serve as a cost-efficient process.

Methods: Mouse BECs (**b.End3**) were cultured in a CELLine AD 1000 bioreactor, and EVs were isolated by sequential centrifugation of culture supernatants.[4,5] We collected both large EVs (**lEVs** > 200 nm) and small EVs (**sEVs** < 200 nm). The sequential centrifugation involved low-speed centrifugation to remove cell debris (300 x g for 11 mins) and ultracentrifugation to pellet lEVs (20,000 x g for 45 mins) and sEVs (120,000 x g for 70 mins). Both EVs were characterized for total EV protein content using micro BCA assay, while dynamic light scattering (**DLS**) was used to measured particle diameter and surface charge. Western blotting was used to identify EV subtype-specific markers. Functional bioactivity of EVs was evaluated using an ATP assay in oxygen-glucose deprived BECs to mimic ischemic stress, and the relative intracellular ATP levels were quantified using luminescence-based ATP assay.[6]

Results: Across three independent isolations, lEVs exhibited larger particle diameters (200 – 230 nm; PDI 0.25 – 0.38), with total EV protein content ranging from (2.6 to 5.3 mg), while sEVs showed smaller particle diameter (75-150 nm; 0.37 – 0.55), with total EV protein content ranging from (2.7 to 5.3 mg). Surface charge measurements confirmed both EV subtypes carried a net negative charge (-25 to -32 mV), consistent with stable colloidal suspensions. Nanoparticle tracking analysis (**NTA**) revealed that particle concentrations of lEVs ranged from $1.28 \times 10^{10} \pm 1.38 \times 10^{10}$ to $7.79 \times 10^{10} \pm 2.1 \times 10^{8}$ particles/mL, whereas sEVs exhibited concentrations ranging from $6.33 \times 10^{10} \pm 3.26 \times 10^{10}$ x $5.22 \times 10^{10} \pm 8.31 \times 10^{8}$ particles/mL. Western blotting confirmed expression of subtype-specific markers, with CD63 being expressed in sEV fraction and Arf6 present in lEVs. Functional assays revealed that both EV subtypes enhanced relative ATP levels in recipient BECs, an EV dose-dependent restoration of ATP levels was noted in sEV-treated BECs compared to lEVs. These effects are attributed to the presence of heat shock proteins in the EV cargo[1], suggesting that bioreactor BEC cultures produce EVs with properties consistent with that of BECs batch-cultured in T-175 tissue culture flasks.

Conclusion: Bioreactor BEC cultures enable scalable, high-yield production of functionally active EVs. These findings highlight their potential for further development for translational therapeutic applications involving metabolic restoration.

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#8

Biodegradable Microparticles Exhibiting Pulsatile Release to Improve Vaccines

Rachakonda R, Kunkel A, McHugh K
Rice University Bioengineering; Rice University Chemistry, Houston, TX

Introduction:
Vaccination remains one of the most powerful tools against infectious disease, preventing approximately 4 million deaths each year.[1] Despite this success, over 14.5 million vaccine-eligible children didn't receive any vaccines in 2023, largely due to factors such as cost and low accessibility in low- and middle-income countries (LMICs), further exacerbated by the need for multiple injections spaced over time.[2] Studies have shown that antigen exposure kinetics that mimic those of a native infection can lead to a more robust immune response, potentially providing better protection against disease. Unfortunately, achieving those kinetics requires tightly spaced repeated administrations or the use of expensive osmotic pumps—both of which are clinically infeasible. Here, we focus on achieving infection-mimicking release kinetics to enhance the immune response using a single injection of microparticles that exhibit pulsatile release after a material-dependent delay using the Particles Uniformly Liquified and Sealed to Encapsulate Drug (PULSED) method. We leveraged the modularity of the PULSED platform to identify poly(lactic-co-glycolic acid) (PLGA) microparticle compositions that can be combined to recapitulate the kinetics of native infections.

Hypothesis: We hypothesize that PULSED microparticles of different polymer blends can achieve intermediate delays relative to their homopolymer counterparts.

Methods:
Particles were fabricated with varying blends of PLGA molecular weights. Microstructures with an open cavity were formed using polydimethylsiloxane (PDMS) molds. PLGA films were placed on top of micropatterned PDMS molds and were heated to 120°C under vacuum for 1 hour. After cooling, molds were separated, resulting in PLGA particles. These hollow, open-faced particles were filled with a fluorescently labeled model drug, then sealed by inversion over a heat source at 200°C for 18 seconds to form a shell around the drug-loaded core. Particles were cooled and harvested using a scalpel. In vitro release kinetics were measured using fluorescently labeled model therapeutics. For the release study, particles were submerged in phosphate-buffered saline and incubated at 37°C with shaking. Supernatant was sampled daily, and fluorescence was measured using a microplate reader.

Results:
We show that blended PLGA microparticles can achieve tunable, pulsatile release to mimic multi-dose drug release kinetics. The blending of different molecular weights of PLGA is successful in achieving release after intermediate delays. PLGA 1 and PLGA 2 demonstrated *in vitro* release after 8+/-1 hours and 7+/-0 days, respectively. Blending PLGA 1 and PLGA 2 at ratios of 70:30 and 15:85 resulted in release after 3+/-1 days and 5+/-1 days.

Conclusions:
Blended PLGA microparticles enable tunable, pulsatile drug release, with intermediate delays achieved by combining polymers of different molecular weights. By combining microparticle populations that release drugs at different times, we can recreate the antigen presentation

patterns of natural infections with a single injection. This approach has the potential to generate a stronger immune response than traditional vaccines, making protection possible after one dose. Our long-term goal is to apply this technology in LMICs to reduce barriers to vaccination and lower the global burden of vaccine-preventable deaths.

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#11

Title: Cell-based therapy for localized brain delivery of dopamine for Parkinson's disease

Authors: Sajedeh Nasr Esfahani[1,2], Thao Nguyen[1], Suki Oji[1], Samantha Fleury[2], Omid Veiseh[2], Robert Krencik[1]

Affiliations: Houston Methodist Research Institute, Rice University

Introduction: Parkinson's disease is the second most common neurodegenerative disorder, characterized by the degeneration of dopaminergic neurons and the resulting loss of dopamine in the brain, which primarily leads to motor dysfunction. Increasing dopamine in the striatum effectively reduces motor dysfunction in Parkinson's disease (PD), with L-DOPA as the standard treatment. However, systemic delivery activates all dopaminergic pathways, causing off-target effects like dyskinesia and non-motor symptoms. Cell therapy offers localized, long-term DA delivery but current approaches face challenges such as poor survival and immune rejection. Building on our expertise in cellular engineering and immune-evasive biomaterials[1], we engineered cells to produce DA and encapsulated them in sodium alginate, an immune-evasive material designed to protect transplanted cells from host immune attack. This "living drug factory" (DopaCapsule) enables localized, sustained, and potentially on-demand DA production, with the goal of improving motor function in PD (**Fig. 1A**).

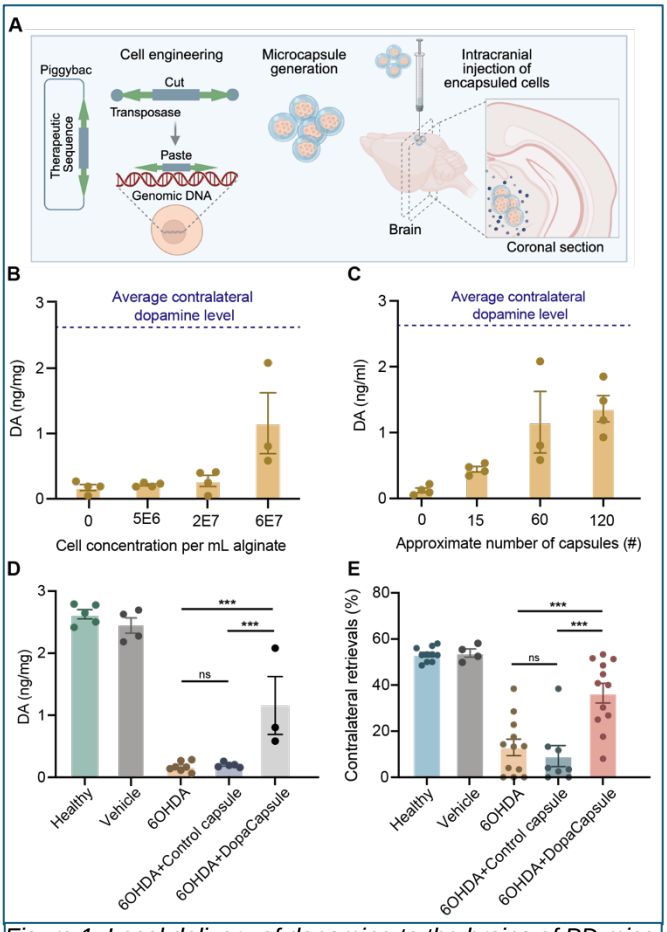

Figure 1. Local delivery of dopamine to the brains of PD mice. A) Schematic of the transplantation procedure. B, C) Modulation of dopamine dosing in PD mice. D) Restoration of striatal dopamine levels following treatment. E) Improvement of behavioral function in PD mice after treatment.

Hypothesis: We hypothesize that transplanting DopaCapsules into the striatum of PD mice will reduce motor dysfunction.

Methods: We successfully engineered adult retinal pigment epithelial (ARPE) cells to produce DA continuously using a piggyBac-based transposase system to express proteins important for DA synthesis (tyrosine hydroxylase, aromatic amino acid decarboxylase, and GTP-cyclohydrolase 1).

Engineered cells were encapsulated within immune evasive biomaterial (DopaCapsule), using an electrostatic droplet generator, to protect the cells from immune cells after transplantation. DopaCapsules were transplanted intracranially into the striatum of PD model mice, generated by unilateral injection of 6-hydroxydopamine (6-OHDA) into the substantia nigra, to enable localized delivery of DA.

Results: Engineered cells successfully synthesized dopamine at a rate of ~0.3 pg/cell/day. DopaCapsules transplanted into the brains of PD model mice remained viable and productive for at least 90 days. By adjusting the number of cells per capsule and the total number of transplanted capsules, DA dosing in the brain could be modulated (**Fig. 1B, 1C**). Enzyme-Linked Immunosorbent Assay (ELISA) measurements confirmed partial restoration of DA levels in the striatum of 6-OHDA–lesioned mice following DopaCapsule transplantation (**Fig. 1D**). Behavioral assessment using the corridor test demonstrated significant sensorimotor improvement in treated animals (**Fig. 1E**).

Conclusion(s): In this study, we successfully engineered cells to synthesize dopamine and encapsulated them in sodium alginate to create DopaCapsules. Transplantation of DopaCapsules into the brains of Parkinson's disease model animals partially restored striatal dopamine levels and improved motor function. This approach highlights the potential of DopaCapsules to advance cell-based therapies for PD, offering a novel strategy for localized, long-term dopamine delivery and paving the way for rigorous preclinical testing.

Key words:

Parkinson's disease, cell-based therapy, encapsulation, dopamine

Acknowledgements:

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#12

A Novel Microbubble-Mediated Approach to Gene Silencing

*Nazia Hafeez[1,2], Sina Khorsandi[1], Nicole McCuen[3], Ruoqi Gao[1], Adam Woodward[1], Shea Garland[1], Prasanna Alluri[3], Jacques Lux[1,2]**
[1]Department of Radiology, Translational Research in Ultrasound Theranostics (TRUST) Program, UT Southwestern Medical Center
[2]Department of Biomedical Engineering, UT Southwestern Medical Center
[3]Department of Radiation Oncology, UT Southwestern Medical Center

Keywords: ultrasound, sonoporation, microbubble, gene silencing, image-guided therapy

Small interfering RNA (siRNA) mediated gene silencing as a form of gene therapy faces drug development issues due to the easily degradable nature and overall negative charge of siRNA1. Our objective is to use targeted microbubbles (MBs) to deliver siRNA into cancer cells to silence genes with spatiotemporal control through the development of a novel platform called "Microbubble-assisted Ultrasound-guided Targeted INhibition of Genes" (MUTING) (**Figure 1**).

MBs were formulated with phospholipid shells and perfluorobutane core. Cationic spermine-modified dextran (SpeDex) and targeting antibodies were thiolated and conjugated onto the MB shells, which was confirmed with flow cytometry. siRNA was loaded onto SpeDex with electrostatic binding to form nanocomplex loaded MBs (ncMBs). Zeta potential and SYBR Gold nucleic acid gel stain confirmed successful siRNA loading onto MBs.

To explore the potential of our platform to successfully deliver siRNA under ultrasound (US) guidance, EpCAM+ 4T1 cells were incubated with ncMBs for 10 min and then insonated for 1 min using a 1 MHz transducer with 2 W/cm2 power density, and 50% duty cycle. To assess MUTING's efficacy in vivo, a 4T1 triple-negative breast cancer model was established in Balb/cJ mice. Tumors were injected intratumorally with controls or ncMBs once they reached ~100 mm^3. For insonation groups, US was applied for 2 minutes at 4 W/cm^2, 50% duty cycle. Tumors were collected 24 hours after treatment for Western blot analysis to assess gene silencing.

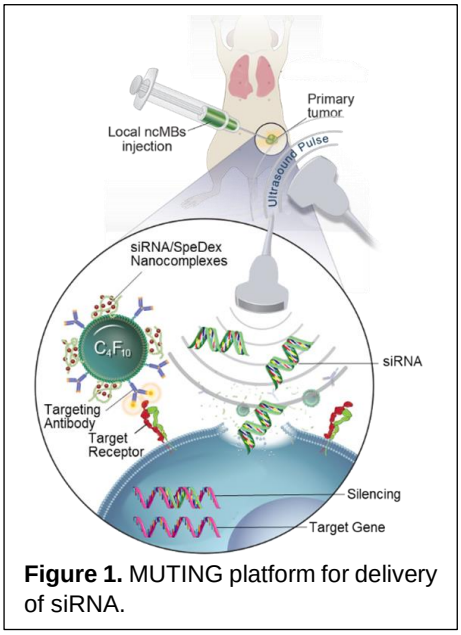

Figure 1. MUTING platform for delivery of siRNA.

MUTING performed better than Lipofectamine transfection against the DNA mismatch repair gene *MLH1* in vitro (**Figure 2a**) and achieved complete silencing in vivo (**Figure 2c**). Additionally, MUTING-mediated silencing of the oncogene c-MYC was successfully achieved in 4T1 cells. (**Figure 2b**). We are now exploring MUTING's potential to target undruggable oncogenes, with the aim of combining it with other therapies for synergistic treatment of cancer.

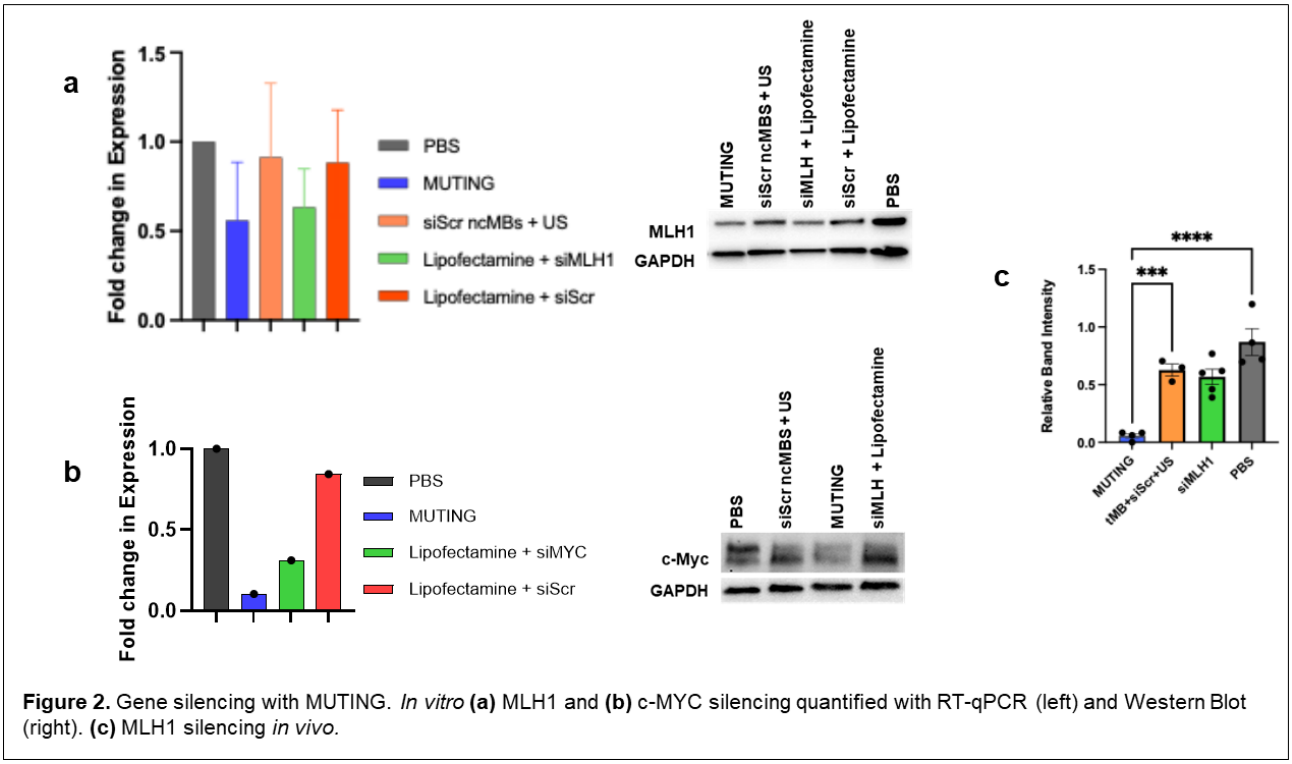

Figure 2. Gene silencing with MUTING. *In vitro* **(a)** MLH1 and **(b)** c-MYC silencing quantified with RT-qPCR (left) and Western Blot (right). **(c)** MLH1 silencing *in vivo*.

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#13 Nanomaterial-Induced Mitochondrial Biogenesis Enhances Intercellular Mitochondrial Transfer Efficiency

Soukar J1, 2, Singh KA2, Aviles A1,4, Hargett S2, Kaur H3, Foster S1, 2, Roy S2, Zhao F2, Gohil VM1, 3, Singh I1, 2, 4, . Gaharwar AK1, 2, 5, *

1-Interdisiplinary program in Genetics and Genomics; 2- Dept of Biomedical Engineering, College of Engineering; 3- Dept of Biochemistry and Biophysics, College of Agriculture and Life Sciences; 4- Dept of Cell Biology and Genetics, College of Medicine; 5- Dept of Material Science and Engineering, College of Engineering, Texas A&M University, College Station, TX 77843, USA

Keywords: Nanoparticles; therapeutics; mitochondrial; tunneling nanotubes; cell-cell communications

Introduction: Intercellular mitochondrial transfer—the spontaneous exchange of mitochondria between cells—is a recently described phenomenon crucial for cellular repair, regeneration, and disease management [1]. Enhancing this natural process holds promise for novel therapies targeting diseases associated with mitochondrial dysfunction. However, despite growing recognition of the therapeutic potential of mitochondrial transfer, its widespread adoption is hindered by inefficient replacement of mitochondria in diseased cells, primarily due to limited rates of mitochondrial translocation [2]. Existing methods to enhance transfer rates—such as overexpressing mechanistic proteins like the motor protein Miro1 and gap junction Cx43, or employed engineering techniques like the isolation of mitochondria, are cumbersome and labor-intensive [3]. Here, we introduce a nanomaterial-based approach employing molybdenum disulfide (MoS_2) nanoflowers with atomic-scale vacancies to stimulate mitochondrial biogenesis in cells to make them mitochondrial biofactories. Upon internalization, these nanoflowers activate the PGC-1α pathway within donor cells, resulting in a twofold increase in mitochondrial mass and enhancing mitochondrial transfer to recipient cells by several-fold.

Hypothesis: To address these limitations, we have developed a biomaterial-based therapeutic strategy employing molybdenum disulfide (MoS_2) nanoflowers with atomic-scale modifications to transform human mesenchymal stem cells (hMSCs) into mitochondrial biofactories. By leveraging these atomic-level modifications to reprogram cellular metabolism, our approach transcends conventional small-molecule therapies, offering a precise, biocompatible, and effective solution. Utilizing nanomaterial platforms allows us to bypass limitations in transfer rates and eliminates the need for complex genetic interventions or extensive use of systemically administered drugs targeting mitochondrial function. This method capitalizes on the natural propensity of MSCs to transfer mitochondria, amplifying this capability through enhanced available mitochondrial mass. These cells transfer more mitochondria to damaged cells, significantly improving energy production and function.

Methods: This *in vitro* study utilizes Flow cytometry, microscopy, cell sorting, and RNA-seq is utilized to uncover mitochondrial movement between cells and fate of recipient cells after mitochondrial donation.

Results: This enhanced efficiency of transfer significantly improves mitochondrial respiratory capacity and adenosine triphosphate (ATP) production under physiological conditions. In cellular models of mitochondrial and cellular damage, MoS_2 enhanced mitochondrial transfer achieved remarkable restoration of cell function. In disease models, this approach restores cell health, offering a new strategy for treating mitochondrial-related disorders. By enhancing the body's own repair mechanisms, this nanomaterial-based method could pave the way for innovative therapies in regenerative medicine.

Conclusions: In this proof-of-concept study we demonstrate that nanomaterial-boosted intercellular mitochondrial transfer can enhance cell survivability and function under diseased conditions, offering a promising new strategy for treating mitochondrial dysfunction-related diseases. Our findings demonstrate that MoS_2 nanoflowers significantly promote mitochondrial transfer between cells, leading to an increased mitochondrial availability within recipient cells. These new mitochondria can be utilized by recipient cells as energy-generating machinery and can integrate into existing mitochondrial networks. Existing approaches to address mitochondrial health and function often lack efficacy and broad applicability, focusing primarily on symptom management. In contrast, our strategy offers a universally applicable solution for various pathologies requiring mitochondrial repair and replacement. By enhancing mitochondrial transfer efficiency, our nanomaterial-based approach introduces MoS_2 nanoflowers as a promising novel agent for promoting mitochondrial biogenesis, thereby providing therapeutic value in management of mitochondrial dysfunction.

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#14

Investigating Targeted Claudin-4 Cross-linked Iron Oxide Nanoworms for the Enhanced MRI Detection of Early-Stage Ovarian Cancer

David Angarita1, Dmitri Simberg1, David Siegel1, Hanmant A. Gaikwad1, Natalie Serkova3, Leah Roseberry3, Benjamin Bilter2, Kian Behbakht2

1-Department of Pharmaceutical Sciences, The Skaggs School of Pharmacy, University of Colorado Anschutz Medical Campus
2-Department of Obstetrics and Gynecology, Division of Gynecological Oncology, University of Colorado Anschutz Medical Campus
3-Department of Radiology, University of Colorado Anschutz Medical Campus

Keywords: Claudin-4, superparamagnetic iron oxide nanoparticles, ovarian cancer, MRI contrast agents, early detection

An ovarian cancer diagnosis remains highly lethal worldwide due to late-stage diagnosis. Seventy-five percent of diagnoses are attributed to ***high-grade serous ovarian carcinoma (HGSC)***, which develops from ***serous tubal intraepithelial carcinoma (STIC)*** precursor lesions in the fallopian tubes. These precursor lesions have a median 7-year lag period before becoming invasive [1,3]. Current screening modalities, including CA125 blood tests, transvaginal ultrasound, and symptom screening, lack the sensitivity and specificity for early HGSC detection, with most diagnoses occurring at stage 3 or higher [3]. Interestingly, **claudin-4 (CLDN4)**, a tight junction protein, is overexpressed in ~80% of HGSC tumors while showing minimal expression in regular fallopian tubes, making it an ideal biomarker for targeted imaging. We previously synthesized 60nm superparamagnetic cross-linked iron oxide nanoworms that are highly stable and have minimal immune recognition. The previously synthesized NWs were fluorescently labeled with Cy5 and conjugated with XX (number of Abs/NP) anti-human Claudin-4 antibodies via click chemistry. We hypothesized that intravenously administered Cy5 anti-CLDN4-CLIO NWs would enable highly sensitive ovarian cancer detection by reducing T2 relaxation times and creating a strong negative contrast (darker) on T2-weighted MRI. In vitro studies evaluated nanoparticle uptake in Claudin-4 positive and negative ovarian cancer cell lines using flow cytometry, confocal microscopy, and quantitative T2-MRI mapping on a 9.4T Bruker scanner. In vitro studies showed >98% specific uptake in Claudin-4 (+) cells with dramatic T2 signal reduction from 195.1ms to 26.6ms compared to controls. In vitro uptake by the Kupffer cell macrophage cell line and primary peritoneal macrophages confirmed low immune uptake. In vivo biodistribution and MRI studies were conducted on two separate cohorts of healthy Balb/c mice and nude mice bearing bilateral OVCAR8 tumors with differential Claudin-4 expression (+/-). Cy5 anti-CLDN4-CLIO NWs demonstrated a 13.4-hour plasma half-life with preferential liver uptake but minimal accumulation in other healthy organs. Spatial immunofluorescence imaging of

tumors stained with multiple markers confirmed higher uptake in Claudin-4 (+) versus Claudin-4 (-) tumors, validating target specificity. However, some non-specific uptake by TAMs and fibroblasts was observed in both Claudin-4 (+) and Claudin-4 (-) tumors, coupled with liver uptake, suggesting the enhanced in vivo immune cell recognition that was not detected via in vitro uptake tests. Current investigations focus on further reducing immune recognition through particle size modification, antibody selection refinement, and complement inhibition. In summary, our Cy5 anti-CLDN4 NWs show promise for detecting precursor lesions during the critical pre-invasive window, but additional optimization in targeting specificity is necessary.

NO DISCLOSURES

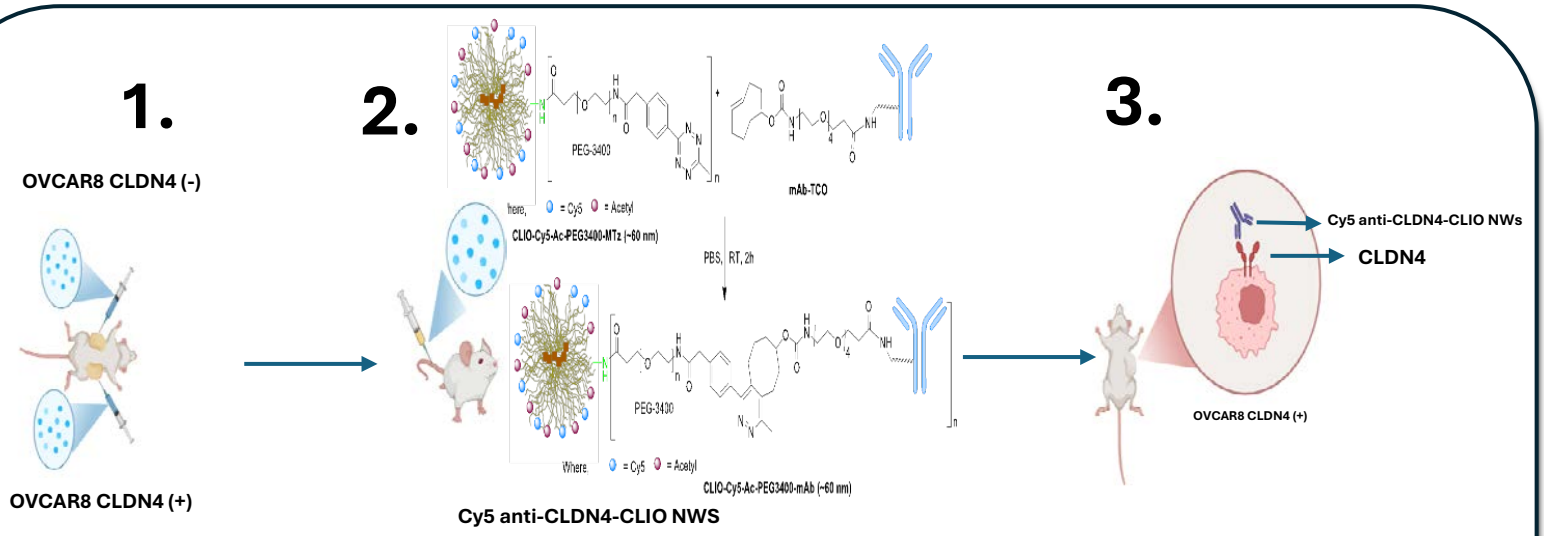

Figure 1. Schematic showing the in vivo model used to demonstrate the tumor targeting specificity of Cy5 anti-CLDN4-CLIO NWs. 1). OVCAR8 cancer cell lines expressing CLDN4 (+) and not expressing CLDN4 (-) were implanted bilaterally on each flank of nude mice. 2) A breakdown of the synthesis of Cy5 anti-CLDN4-CLIO NWs that were then intravenously injected into nude mice. 3) Our proposed hypothesis showing that Cy5 anti-CLDN4-CLIO NWs will selectively bind to tumors expressing CLDN4.

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#15

A Therapeutic Approach for Breast Cancer Liver Metastasis Using Macrophages as a Carrier for Delivering siEphA2 Lipid Nanoparticles: An *In-vitro* and *In-vivo* Study

Anjana Tiwari[1], LaMargaret T. Johnson[2] , Eric Chau[1], Dylan A. Goodin[2], Arvind Pandey[3], Hermann B. Frieboes[2,4,5,,6,*] and Biana Godin[1,7,8,*]

[1] Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX USA

[2] Department of Bioengineering, University of Louisville, Louisville, KY, USA

[3] Department of Neurosurgery, Houston Methodist Research Institute, Hoston, TX, USA

[4] Department of Pharmacology/Toxicology, University of Louisville, Louisville, KY USA

[5] UofL Health – Brown Cancer Center, University of Louisville, Louisville, KY, USA

[6] Center for Predictive Medicine, University of Louisville, Louisville, Louisville, KY, USA

[7] Department of Obstetrics and Gynecology, Houston Methodist Hospital, Houston, TX, USA

[8] Department of Biomedical Engineering, Texas A&M University, College Station, TX, USA

Introduction: Breast cancer is the most frequent malignancy in women worldwide. Liver is one of the most common locations for breast tumor dissemination and breast cancer liver metastases (BCLM) account for the highest mortality rate in patients. Unlike primary tumors and metastatic lesions in other organs, BCLM growth relies heavily on the liver vasculature, leading to lesions that are hypo-perfused. Consequently, most BCLM clinically appear as hypoattenuating spots. This lack of perfusion prevents systemically administered agents from reaching the tumor core, thus causing physiological, transport-related resistance to therapy. The liver is an organ enriched in macrophages, which play important roles by affecting tumor growth, angiogenesis, metastasis and chemoresistance. These phagocytic cells are localized in proximity to BCLM and have been clinically utilized for imaging with nanomaterials. Macrophages influence the local immune response by releasing factors that aid tumor progression (M2 phenotype) or its inhibition (M1 macrophages). Our previous studies have shown that drug transport barriers in BCLM can be overcome using macrophages as carriers for nanotherapeutics, leading to efficient therapy for hypovascularized lesions in the liver. When drug is retained in the cellular milieu in the proximity of the tumor lesion, sufficiently high local concentrations of therapeutics can be obtained to kill the tumor cells. The prerequisite for this therapy is the ability of BCLM to attract macrophages from surrounding liver tissue.

Methods: We utilized 2D and 3D co-cultures to study the recruitment of macrophages by a variety of human breast cancer cells, including, MCF7 (ER+, PR+/-, HER2-), T-47D (ER+, PR+/-, HER2-), MDA-MB-231 (triple negative breast cancer, TNBC), SUM-159 (TNBC) for the purpose. We determined the macrophage infiltration using a) confocal (static) and Incucyte (kinetic) microscope imaging, b) transwell cell migration assay for cell number, c) immunofluorescence. We designed, characterized and evaluated the effect of siEphA2 loaded lipid nanoparticles (siEphA2-LNP) in combination with albumin bound paclitaxel (nAb-PTX) on macrophage phenotype, transport and anti-cancer efficacy *in vitro* and *in vivo* BCLM models.

Results: The siEphA2-LNP and nAb-PTX treated macrophages shifted the TME towards the pro-inflammatory, anti-tumorigenic milieu by polarizing the macrophages from the M2 to M1 phenotype. This resulted in increased apoptosis and decreased proliferation in MCF7 and MDA-MB-231 human breast cancer cells in treated groups as compared to controls. Studies in the 3D *in vitro* model that mimics clinically observed hypovascularized BCLM surrounded by

macrophages, suggested that a balance between M1 and M2 macrophage phenotypes in the TME is required to optimize nanotherapeutic efficiency. *In-vivo* data in two different models of liver metastasis of triple negative breast cancer confirm the efficacy of the combination treatment (siEphA2-LNP and nAb-Paclitaxel) in reducing the metastatic burden.

Conclusions: Our study provides a rationale for utilizing macrophages as a depot for nanotherapeutics in BCLM microenvironment. This technique could be utilized to design improved nanocarriers for the therapy of BCLM and other hypo-perfused tumors. Altogether, this study shows that EphA2 siRNA-loaded lipid nanoparticles can decrease BCLM tumor burden by modulating the tumor microenvironment.

#16

PAS domain length governs ferritin nanocage stability and enables PAS40-based nanotracers for fluorescence-guided surgery

Valeria Giacobbo (1), Ilaria Tagliolini (1), Francesca Gorgoglione (1), Marta Sevieri (1), Beatrice Bignami (1), Arianna Bonizzi (1), Claudia Pigliacelli (2), Francesca Baldelli Bombelli (2), Fabio Corsi (1,3), Renata Tisi (4), Serena Mazzucchelli (1)

1 - Nanomedicine Laboratory, Dept of Biomedical and Clinical Sciences, University of Milan, Milan, Italy
2 - Dept of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy
3 - Breast Unit, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy
4 - Dept of Biotechnology and Biosciences Università di Milano-Bicocca, Milan, Italy
Keywords: Drug delivery, H-Ferritin nanocages, Nanoparticles, Protein nanocages, Cancer

Ferritin nanocages (HFn) present a promising approach for targeted drug delivery to cancer cells and tumor detection, thanks to their unique structure and natural tumor-homing properties (1). Despite their promising potential, HFn nanoparticles suffer from short half-life and rapid clearance. To overcome these issues, PASylation has been used without introducing negative effects (2), but the effect of PAS domain length remains unclear. Here, we compared HFn nanoparticles modified with 20 amino acid PAS domains (HFn-PAS20) or 40 amino acid PAS domains (HFn-PAS40). While HFn-PAS20 monomers could be produced and purified, molecular simulations revealed that shortened PAS domains destabilized higher-order assemblies by disrupting C-terminal E-helix interactions. In contrast, HFn-PAS40 formed stable nanocages, supporting the necessity of longer PAS chains for correct quaternary organization. Building on these findings, we loaded HFn-PAS40 with the near-infrared dye indocyanine green (ICG) to generate HFn-PAS40-ICG nanotracers for fluorescence-guided surgery (3). Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and Circular Dichroism (CD) confirmed the successful production of HFn-PAS40 nanocages with consistent size and morphology. Then, ICG was encapsulated into the nanocages to form the HFn-PAS40-ICG complex through a pH-dependent method. The *in vitro* behaviour of HFn-PAS40-ICG was evaluated in 2D cell cultures using 4T1-Luc murine breast cancer (BC) cells, and in 3D cell cultures using patient-derived organoids (PDOs). *In vitro* experiments showed that PASylation inhibited TfR1-mediated interactions with cancer cells, which were restored following PAS removal. The HFn-PAS40-ICG performances *in vivo* were evaluated in murine models of breast and colorectal cancer. HFn-PAS40-ICG exhibited significantly higher fluorescence accumulation at the tumor site in a breast cancer model, with a 3-fold higher signal compared to the control. Similar results were observed in the colorectal cancer model. Together, these results demonstrate that PAS domain length is critical for HFn stability, and that HFn-PAS40 offers a versatile platform for tumor-specific imaging and fluorescence-guided surgery.

Acknowledgements: This work was supported by AIRC - Associazione Italiana per la Ricerca sul Cancro (IG2022_ID27107 P.I Mazzucchelli).

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#17

Codon optimality modulates cellular stress and innate immune responses triggered by exogenous RNAs

Chotiwat Seephetdee[1,2,3,4#,12] Nada Bejar[1,2,3,12], Eric Chau[5,12], Thy Y. Nguyen[5,12], Biana Godin[3,5,6,7,8,9,10,11,12], and Daniel L. Kiss[1,2,3,10,11,12#]

[1]Center for RNA Therapeutics, [2]Department of Cardiovascular Sciences, [3]Houston Methodist Academic Institute, [4]Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand, [5]Department of Nanomedicine, [6]Department of Obstetrics and Gynecology [7]Department of Obstetrics and Gynecology, Weill Cornell Medicine College, New York, NY, USA, [8]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Sciences Center, Houston, TX, [9]Department of Biomedical Engineering, Texas A&M, College Station, TX, [10]Weill Cornell Medical College, [11]Houston Methodist Neal Cancer Center, [12]Houston Methodist Research Institute, 6670 Bertner Ave, Houston, TX 77030 USA

Keywords: RNA therapy, Codon optimization, Induced Stress, mRNA therapeutics

Abstract:

Despite advancements in RNA sequence design, evidence regarding the preferential use of synonymous codons on cellular stress and innate immune responses is lacking. To this end, we developed a new codon optimality formula to re-engineer the coding sequences of three luciferase reporters. We demonstrate that mRNAs enriched in optimal codons elicited dramatic increases in luciferase activities compared to less optimal sequences both *in vitro* and *in vivo*. Notably, transfecting low optimality test RNA suppresses the translation of co-transfected control mRNAs in dual reporter assays. Further, low optimality mRNAs activated innate immune pathways and the phosphorylation of the translation initiation factor eIF2α, a central event of the integrated stress response. eIF2α phosphorylation was suppressed by a GCN2 inhibitor, but not by other eIF2α kinase inhibitors. Using nucleoside-modified or circular RNAs also fully or partially abrogated these responses. Finally, optimal, but not non-optimal, circular RNAs have enhanced RNA lifespan and duration of protein expression. Our results show that RNA sequence, composition, and structure all govern RNA translatability. Further, RNA sequences with poor codon optimality are immunogenic and induce cellular stress. Together, we show that RNA coding sequence design is a key consideration for both mRNA and circular RNA therapeutics.

#18

DiPhyPC is a novel candidate lipid to enhance LNP stability and efficacy

Rei J. Abe (1,2), Eric Chau (3), Karem Court (3), Biana Godin (3,4), Henry Pownall (1,4)
1. Department of Bioenergetics/Medicine, Houston Methodist Research Institute, Houston, TX
2. Department of Physiology, Biophysics, and Systems Biology, Weill Cornell Graduate School, New York, NY
3. Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
4. Weill Cornell Medicine, New York, NY, USA

Keywords: diphytanoyl phosphatidylcholine (DiPhyPC), reconstituted HDL (rHDL), LNP stability, biodegradation

Introduction: Lipid nanoparticles (LNPs) are used as clinical drug delivery systems for nucleic acids. During the COVID-19 pandemic, LNPs saw widespread use through mRNA vaccines and several clinical trials are currently evaluating the value of LNP-based siRNA and mRNA therapeutics and vaccines for other pathological disorders. Intravenous (IV) administration is a common route for administration of nucleic acid based therapeutics; furthermore, for specific disease settings such as atherosclerotic cardiovascular disease (ASCVD), IV administration is preferred to target atherogenic endothelial regions within the circulation. Despite their clinical use, pharmacokinetic studies of several lipid species in mice and humans have suggested that most phospholipid (PL)-based LNPs are vulnerable to in vivo physico-chemical and physiological challenges that lead to biodegradation. PL, essential lipid components within cell membranes and lipoproteins, impart PL-based LNPs biocompatibility. However, PL are also susceptible to spontaneous lipid transfer, hydrolase activities, and extraction by lipoprotein receptors; as a result, PL-based LNPs are unstable in plasma, resulting in a reduction in LNP efficacy. Diphytanoyl phosphatidylcholine (DiPhyPC), a PC with nonhydrolyzable bulky acyl chains, lacks double bonds making it oxidation resistant. PCs with large hydrocarbon chains transfer slowly and as a result, DiPhyPC is expected to have a long spontaneous desorption half-life.

Hypothesis: We hypothesize that DiPhyPC is a novel candidate-PC for enhanced LNP stability and therapeutic efficiency in IV-LNP administration relative to traditional LNP phospholipids.

Methods: Reconstituted HDL (rHDL) is used as a model to isolate and examine PL-dependent stability. This approach eliminates the confounding effects of other lipids, such as ionizable and cationic lipids, that are typically incorporated in LNPs, allowing for a focused analysis of PL contributions. ^{14}C-DiPhyPC was chemically synthesized from Diphytanoyl phosphatidylethanolamine (DiPhyPE), while ^{3}H-distearoyl phosphatidylcholine (DSPC) was purchased commercially and used as a traditional LNP-PL control. These PCs were then used as a radiolabel during the detergent reconstitution of HDL using sodium cholate. Radiolabeled rHDL were then incubated with hypothetical physico-chemical LNP-PL disruptors to assess the potential for DiPhyPC to improve LNP stability.

Results: We have discovered that traditional LNP formulations are susceptible to biodegradation by plasma lipases, and that DiPhyPC transfer rates are slower than those of conventional PCs.

Our results demonstrate that DiPhyPC spontaneous transfer rates are slower from rHDL to LDL relative to DSPC. Similarly, rHDL-DiPhyPC uptake via scavenger receptor class B type 1 (SRB1), a prominent hepatic lipoprotein receptor is slower relative to DSPC.

Conclusion: The clinical potential of LNPs is limited by their biodegradation when administered intravenously. Validation of optimized LNP designs as superior IV delivery vehicles will provide compelling rationale for future tests of their efficacy in the treatment of multiple diseases that require LNP survival in the plasma compartment.

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#19

A Novel circRNA vaccine platform for Chikungunya is more effective than linear mRNA

Daniel L. Kiss[1,2,3], Nada Bejar[1,2,3], Daniel R. Boutz4[3,4,5], Murilo T.D. Bueno[1,3], Kathrina Castillo[1,2,3], Tulsi Ram Damase[1,3], Wataru Horikawa[1,2,3], Andrew P. Horton[3,4,5], Shaunak Kar[3,4,5], Sana Koubaa Kharrat[1,2,3], Hongyi Li[1,3], Chiara Mancino[3,6,7], Amanda Mareth[8,9], Jessica A. Plante[8,9], Ankita Rao[1,2,3], Rachel Reyna[8,9], Chotiwat Seephetdee[1,3,10], Thomas Segall-Shapiro[3,4,5], Vrutant V. Shah[1,2,3], Raghav Shroff[3,4,5], Trinh T. Tat[1,2,3], Xiaoxiao Wang[1,3], Qing Yan[1,3], Kristopher W. Brannan[1,2,3], Jimmy Gollihar[3,4,5], Kenneth Plante[8,9], Francesca Taraballi[3,6,7], Scott Weaver[8,9], John P. Cooke[1,2,3]

1. Center for RNA Therapeutics, Houston, United States. 2. Department of Cardiovascular Sciences, Houston, United States. 3. Houston Methodist Research Institute, Houston, United States. 4. Center for Infectious Diseases, Houston, United States. 5. Antibody Discovery & Accelerated Protein Therapeutics (ADAPT) Laboratory, Houston, United States. 6. Center for Musculoskeletal Regeneration, Houston, United States. 7. Orthopedics and Sports Medicine, Houston, United States. 8. World Reference Center for Emerging Viruses and Arboviruses, Galveston, United States. 9. University of Texas Medical Branch, Galveston, United States. 10. Department of Microbiology and Immunology, Faculty of Science, Mahidol University, Bangkok, Thailand

Introduction & Objectives

mRNA vaccines have proven remarkably effective, but circular RNA (circRNA) vaccines have yet to enter the market. Funding from the Coalition for Epidemic Preparedness and Innovation galvanized advances in our antigen, RNA, and nanoparticle design and production for proof-of-concept testing in well-established chikungunya models.

Materials & Methods

We used machine learning to predict stabilizing mutations in the chikungunya virus (CHIKV) structural protein and validated antigen designs using a high throughput mammalian surface display assay. The three most promising antigen sequences were incorporated into our novel circRNA platform, and the candidates were evaluated in vitro. To maximize antigen production, we used both standard reporter assays and ribo-STAMP (Surveying Targets by APOBEC-Mediated Profiling) to screen a matrix of over 20 internal ribosome entry site (IRES), codon optimality formula, and open reading frame combinations across several cell lines. circRNAs encoding the three promising designs of the CHIKV structural protein were manufactured, cell surface expression validated, and circRNAs were encapsulated into ionizable lipid-based nanoparticles. The sequence of the circRNA was verified by a new rolling circle library preparation protocol and the encapsulated drug product was characterized and construct integrity was confirmed by standard assays. The three LNP-encapsulated candidates were injected into IFNAR$^{-/-}$ C57Bl/6 mice. For each test group, 10 animals (5 male, 5 female) were injected with either diluent, LNP-circRNA [10 µg; either day 0 (prime only) only or day 0 + day 28 (boost)], or a single dose of a live attenuated chikungunya virus vaccine as a control. Animals were monitored and blood draws (Days 7, 28, and 56) were assayed with plaque-reduction neutralization tests.

Results

Significant immune responses were observed after the prime, with major increases after the boost with all circRNAs tested. Next, the most consistent candidate was selected for a dose de-escalation study where IFNAR$^{-/-}$ C57Bl/6 mice (5 per group) were injected with either 10, 5, or 1 µg of circRNA or 10, 5, or 1 µg of modified linear mRNA with the identical antigen sequence using the same prime only or a prime + boost (and blood draw) schedule described above. Animals were challenged with 10^4 PFU of the La Reunion strain of CHIKV administered via footpad injection and monitored for 3 weeks. For the prime + boost regimen, both circRNA and mRNA were fully protective at higher doses, but only circRNA offered partial protection at the lowest dose. Furthermore, the circRNA candidate offered better protection than linear mRNA for the prime-only regimens.

Conclusion

With these promising data, we manufactured a cGMP-quality test lot of our circRNA candidate and began regulatory discussions prior to a non-human primate trial to begin in November. To conclude, by using a lethal challenge model, we provide in vivo proof of concept data for our novel circRNA vaccine platform and show that we have successfully generated a novel and effective circRNA vaccine against CHIKV. Further, as judged by multiple metrics in our lethal challenge trial, our circRNA platform is more effective in dose-sparing efficacy than modified mRNA.

#22

Prophylactic and therapeutic cancer vaccine with continuous localized immunomodulation

Kota N[1], Gonzalez DD[1], Liu HC[1], Viswanath D[1], Vander Pol R[1], Wood A[1], Di Trani N[1], Chua CYX[1], Grattoni A [1, 2, 3]

[1] Department of Nanomedicine, Houston Methodist Research Institute;
[2] Department of Surgery, Houston Methodist Hospital;
[3] Department of Radiation Oncology, Houston Methodist Hospital

Abstract:
Selective in vivo immune cell manipulation offers a promising strategy for cancer vaccines. In this context, spatiotemporal control over recruitment of specific cells, and their direct exposure to appropriate immunoadjuvants and antigens are key to effective cancer vaccines. We present an implantable 3D-printed cancer vaccine platform called the 'NanoLymph' that enables spatiotemporally-controlled recruitment and manipulation of immune cells in a subcutaneous site. Leveraging two reservoirs each for continuous immunoadjuvant release or antigen presentation, the NanoLymph attracts dendritic cells (DCs) on site and exposes them to tumor-associated antigens. Upon local antigen-specific activation, DCs are mobilized to initiate a systemic immune response. NanoLymph releasing granulocyte-macrophage colony-stimulating factor and CpG-oligodeoxynucleotides with irradiated whole cell tumor lysate inhibited tumor growth of B16F10 murine melanoma in a prophylactic and therapeutic vaccine setting. Overall, this study presents the NanoLymph as a versatile cancer vaccine development platform with replenishable and controlled local release of antigens and immunoadjuvants.

#23

Title: Development and Characterization of a Biodegradable Nanofluidic Drug-Eluting Seed for Sustained Intratumoral Drug Delivery of Immunotherapeutic Agents

Authors: Francesco Manfredi[1,2], Jingyi Wang[1,3], Eleonora Molinari[1,4], Robin Vander Pol[1], Casey Lewis[1], Xinyi Peng[1], Nicola Di Trani[1], Marco Maria Paci[1,2], Danilo Settis[1,4], Madison Alexandra Deeson[1,5], Yongbin Liu[1], Michael Ittmann[6], Dinh Chuong Nguyen[7], Simbarashe Jokonya[7], Patrick S. Stayton[7], Corrine Ying Xuan Chua[1,#], Alessandro Grattoni[1,8,9, #]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA
[2]College of Engineering, Swansea University, Swansea, UK
[3]Department of Nuclear Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[4]Department of Mechanical and Aerospace Engineering, Polytechnic of Turin, Turin 10129, Italy
[5]Texas A&M University Naresh K. Vashisht College of Medicine, Bryan, TX 77807, USA
[6]Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA
[7]Department of Bioengineering and Molecular Engineering & Science Institute, University of Washington, Seattle, WA, USA
[8]Department of Surgery, Houston Methodist Hospital, Houston, TX 77030, USA
[9]Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX 77030, USA
#Corresponding authors

Abstract

Introduction: Intratumoral immunotherapy offers a promising strategy to improve cancer treatment; however, heterogeneous drug distribution within the tumor and rapid drug leakage limit its therapeutic potential.

Hypothesis: To address these challenges, we developed the biodegradable Nanofluidic Drug-Eluting Seed (b-NDES), an implantable device designed to enhance intratumoral immunotherapy delivery, minimize systemic dissemination, and biodegrade over time, eliminating the need for surgical removal.

Methods: Four formulations with varying ratios of polycaprolactone (PCL), poly(lactic-co-glycolic acid) (PLGA), and barium sulfate were electrospun to fabricate the implants. Surface modifications were implemented to tailor drug elution rates. Morphology, in vitro release of a CD40 agonist antibody, and degradation profiles were evaluated to identify the optimal formulation. Devices were then implanted intratumorally in a 4T1 triple-negative breast cancer (TNBC) murine model using a trocar-based procedure to assess CD40 agonist antibody biodistribution and therapeutic efficacy when co-delivered with a STING agonist and combined with radiotherapy.

Results: The resulting devices featured a hollow cylindrical drug reservoir with a nanofibrous wall, where interconnected fibers formed fine pores enabling passive drug diffusion. Surface modification induced partial fusion of the nanofibers, reducing porosity and thereby regulating release kinetics. Among all tested formulations, the 1:4 PCL:PLGA with barium sulfate exhibited the most sustained and consistent in vitro release together with the fastest degradation rate, and was therefore designated as the final composition for b-NDES and selected for in vivo studies. In 4T1 TNBC murine models, b-NDES enabled localized and prolonged delivery of the CD40 agonist antibody, which remained confined within the tumor. Notably, complete tumor eradication was achieved when b-NDES-mediated delivery of the CD40 agonist antibody and the STING agonist was combined with radiotherapy, without evidence of systemic toxicity.

Conclusion: These findings establish b-NDES as a minimally invasive intratumoral platform capable of localized, controlled, and sustained drug delivery, ideally suited for combination therapies in the treatment of aggressive tumors such as TNBC.

Acknowledgements: This study was funded by the Houston Methodist Research Institute and the Nancy Owens Breast Cancer Foundation (Alessandro Grattoni and Corrine Ying Xuan Chua).

#24

Renal Clearable Iron Oxide Nanoparticles for Potential Dual Modality Imaging of T1-weighted MRI and PET

Pohlee Cheah[a], Liying Sun[b], Jing Qu[a], Dexing Zeng[b], Yongfeng Zhao[a]
[a] Department of Chemistry, Physics and Atmospheric Science, Jackson State University, Jackson, MS, 39217
[b] Center of Radiochemistry Research, Oregon Health & Science University, Portland, OR 97239

Keywords: iron oxide nanoparticles, renal clearance, radiolabeling, magnetic resonance imaging, positron emission tomography

Introduction: Superparamagnetic iron oxide nanoparticles (SPIONs) have recently emerged as promising nanotheranostic agents. However, these SPIONs are typically used as T_2-weighted magnetic resonance imaging (MRI) contrast agent and are primarily cleared by the liver. This clearance raises their long-term safety concerns and repeated injections in MRI applications.

Hypothesis: We hypothesized that size-controlled and highly colloidally stable SPIONs can be engineered for both T_1- and T_2-weighted MRI. We further investigated how nanoparticle size influences biodistribution, with an emphasis on promoting renal clearance.

Methods: Water dispersible iron oxide nanoparticles were synthesized via thermal hydrolysis in polyols. Biodistribution and renal clearance were studied after radiolabeling the nanoparticles with ^{64}Cu.

Results: We successfully synthesized ultrasmall SPIONs with tunable sizes (3–12 nm) through rapid thermal hydrolysis and reduction of iron(III) chloride ($FeCl_3$) in diethylene glycol under basic conditions. The size-controlled SPIONs exhibited dual imaging potential: smaller particles showed properties favorable for T_1-weighted MRI, whereas larger ones functioned effectively as T_2 agents. Cytotoxicity assays in HepG2 cells confirmed good biocompatibility up to 50 μg Fe/mL. Additionally, the SPIONs demonstrated drug-loading capacity by electrostatically binding the anticancer drug metformin, as verified by UV–Vis and FTIR analyses. The nanoparticles were also successfully radiolabeled with ^{64}Cu, enabling PET imaging. In vivo biodistribution and PET studies confirmed efficient renal clearance and highlighted their potential as effective cancer imaging probes.

Conclusions: This study demonstrates that size-tunable, colloidally stable SPIONs possess excellent biocompatibility and efficient renal clearance. Their versatile surface functional group enables both drug-loading and radiolabeling. These features make them strong candidates for dual modality imaging and imaging-guided therapy using T_1-weighted MRI and PET imaging.

Acknowledgements: This research was supported by the National Science Foundation (NSF)(Grant No: DMR-2144790)

#25

Novel Platinum Nanotherapeutics Reprogram the Tumor Microenvironment to Potentiate Cancer Immunotherapy

Yongbin Liu, PhD[1], Xueying Ge, PhD[2], Dongfang Yu, PharmD[1], Junjun Zheng, PhD[3], Roderic I Pettigrew, PhD, MD[2], Shu-Hsia Chen, PhD[4], Junhua Mai, PhD[1,3]

[1]Department of Nanomedicine, Houston Methodist Academic Institute, Houston, TX
[2]School of Engineering Medicine/ENMED, Texas A&M University and Houston Methodist Hospital, Houston, TX
[3]Immunomonitoring Core, Center for Immunotherapy Research, Houston Methodist Research Institute, Houston, TX
[4]Center for Immunotherapy and Neal Cancer Center, Houston Methodist Academic Institute, Houston, TX

Keywords: Platinum; Tumor Microenvironment; Immunotherapy; Nanotherapeutics; Reactive oxygen species

Introduction: Immune checkpoint inhibitors (ICIs)-based immunotherapy has transformed cancer treatment, but its efficacy is often limited by immune suppressive cells in the tumor microenvironment (TME), such as tumor-associated macrophages (TAMs). We recently developed a novel platinum therapeutic (Carrier-Pt) that can induce cancer cell death through triggering rapid and robust intracellular reactive oxygen species (ROS) storm. Interestingly, ROS production was found to enhance anti-cancer immunity by inducing immunogenic cell death (ICD) and reprograming immunosuppressive TAMs. Thus, we aim to investigate its underlying mechanisms and potential synergy with immunotherapy.

Hypothesis: We hypothesize that carrier-Pt will induce ICD in cancer cells and repolarize TAMs to the M1-like phenotype *in vivo*, thereby enhancing the effectiveness of ICIs, leading to sustained immune activation and cancer eradication.

Methods: CT26 and PyMT-N cells were inoculated into immunocompetent and immunodeficient nude mice, and tumor growth was compared. Immune cell populations were analyzed by imaging mass cytometry (IMC). CT26 cells were treated with Carrier-Pt to assess ICD markers (CRT by flow cytometry, ATP by luminescence), while bone marrow–derived macrophages were evaluated for CD80/CD206 (flow cytometry) and TNF-α (ELISA). CT26 tumor–bearing mice received Carrier-Pt alone or in combination with anti–PD-1 therapy, and tumor growth was monitored to evaluate therapeutic efficacy.

Results

Carrier-Pt at 4 mg(Pt)/kg significantly inhibited CT26 and PyMT-N tumor growth compared with the untreated group in both immunocompetent and immunodeficient nude mice ($P < 0.0001$). Notably, in immunocompetent mice, 3 out of 10 animals became tumor-free, whereas in immunodeficient nude mice, all tumors continued to grow, although at a slower rate, after Carrier-Pt treatment. Carrier-Pt administration significantly increased the infiltration of $CD8^+$ T cells and macrophages in CT26 tumors ($P < 0.05$), with Pt signals predominantly enriched in macrophage and fibroblast populations within TME ($P<0.05$). Notably, carrier-Pt treatment also showed a trend toward increasing the frequency of $CD8^+PD\text{-}1^+$ T cells. M2 macrophages treated with Carrier-Pt exhibited a marked upregulation of TNF-α and CD80 ($P < 0.0001$), indicating a shift toward the M1 phenotype. Additionally, CT26 cells exposed to Carrier-Pt displayed elevated surface exposure of calreticulin and ATP release ($P < 0.0001$), indicative of ICD. Finally, the combination of Carrier-Pt with anti–PD-1 therapy synergistically suppressed CT26 tumor growth compared with either treatment alone.

Conclusion:

Carrier-Pt can reprogram the TME by repolarizing immunosuppressive TAMs toward an M1 phenotype and inducing ICD of tumor cells, thereby increasing $CD8^+$ T cell infiltration and enhancing the efficacy of anti-PD-1 immunotherapy.

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Conflicts of Interest
Yongbin Liu and Dongfang Yu are the inventors for the patent that describes carrier-platin and amino acid polymer-platinum anticancer drug conjugates. The other authors declare no conflict of interest.

#26

TITLE: Machine Learning-Integrated Physiology-Based Pharmacokinetic Modeling for Optimizing Nanoparticle Design

Joseph Cave [1,2], Carmine Schiavone [1,3], Zhihui Wang [1,4], Vittorio Cristini [1,4], Prashant Dogra [5]

[1] Mathematics in Medicine Program, Houston Methodist Research Institute, Houston, TX

[2] Dept of Physiology and Biophysics, Weill Cornell Medical College (WCMC), New York, NY, USA

[3] Dept of Chemical, Materials, and Industrial Production Engineering, Univ of Naples Federico II, Naples, Italy

[4] Physiology, Biophysics, and Systems Biology Program, Graduate School of Medical Sciences, WCMC, New York, NY, USA

[5] Titus Family Department of Clinical Pharmacy, University of Southern California, Los Angeles, CA, USA

OBJECTIVES

Since the advent of liposomal Doxorubicin (DOXIL®) as a clinical cancer treatment, nanomedicine has advanced significantly, allowing for precise control over nanoparticle (NP) properties such as size, morphology, zeta potential (ζ), surface chemistry, and composition[1]. Yet, despite these advances, clinical translation of nanoparticle (NP) therapeutics remains limited[2], largely due to nano–bio interactions that drive rapid clearance by the liver and spleen. These processes reduce tumor accumulation and raise safety concerns from unintended off-target exposure. To address this, we developed a physiologically based pharmacokinetic (PBPK)–integrated machine learning (ML) framework that predicts organ-specific biodistribution trajectories directly from NP physicochemical properties and experimental conditions. This framework is designed to support the rational design of NPs with optimized bioaccumulation profiles.

METHODS

We curated a literature-derived dataset of 790 NP biodistribution studies in healthy and tumor-bearing mice following single intravenous injection, encompassing diverse physicochemical properties and experimental settings. To harmonize heterogeneous data (incomplete organ coverage, variable timepoints, inconsistent quality), each dataset was fitted to a minimal eight-compartment PBPK model[3] (plasma, liver, spleen, lungs, kidneys, heart, tumor, clearance) using MATLAB's *ode23s* solver. Fits were evaluated via visual predictive checks and Pearson correlation, yielding 492 standardized concentration–time profiles sampled at eight consistent timepoints.

For each organ, standardized trajectories were clustered using K-Means with Dynamic Time Warping (DTW). Hyperparameters (cluster number, Sakoe–Chiba radius, initialization replicates) were optimized by grid search maximizing the Calinski–Harabasz index. Organ-level neural networks were then trained to predict trajectory class from NP design features, using 10-fold cross-validation with stratified 80/20 splits. Hyperparameters (depth, units, activations, regularization, dropout, learning rate) were tuned via *Keras-Tuner* with early stopping. Model performance was evaluated using PR-AUC-Macro (three-cluster organs) or PR-AUC (two-cluster organs), and interpretability was assessed using *SHAP* to quantify structure-activity relationships.

RESULTS

- **Literature survey:** We assembled the largest harmonized preclinical NP biodistribution dataset to date, covering 790 murine studies and spanning 12 physicochemical design features (e.g., NP class, composition, size, ζ-potential, surface modifications, shape) and 4 experimental variables (e.g., tumor model, assay method).
- **PBPK screening:** PBPK fitting produced 492 high-quality standardized organ-level profiles. Of these, 387 studies reported ≥3 organ compartments, supporting robust PBPK calibration and inclusion in model training. 105 studies contained ≤2 compartments and were retained as an external test set.
- **Trajectory clustering:** DTW-based clustering identified reproducible biodistribution patterns. Plasma, liver, kidney, and heart exhibited three distinct trajectories, whereas spleen, lung, and tumor followed two. Intra-organ clusters reflected ADME differences, primarily distinguished by clearance half-life ($T_{1/2}$) and peak concentration (C_{max}).
- **Predictive modeling:** Organ-specific neural networks achieved strong predictive accuracy. For three-cluster organs, PR-AUC-Macro exceeded 0.75; for binary-cluster organs, PR-AUC exceeded 0.90. Performance was consistent across folds, demonstrating model stability and generalizability.
- **Feature attribution:** SHAP analysis highlighted NP size, composition, and assay type as dominant predictors across organs. ζ-potential, species, and targeting ligand were particularly influential for tumor trajectory classification.

CONCLUSION

We introduce a robust, interpretable ML–PBPK pipeline that predicts organ-specific nanoparticle kinetics from design features. By uncovering reproducible trajectory classes and key design determinants, this approach provides a data-driven tool to rationalize NP development. The framework offers translational potential for optimizing NP design toward improved tumor delivery and reduced off-target exposure.

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#27

Reverse Engineering Electrogenetic Interfaces: Toward Programmable Bioelectronic Systems for Cell Therapy

Authors:

1. Jaskirat Singh Batra, Postdoctoral Fellow, Nanomedicine, Houston Methodist Research Institute, TX
2. Marco Maria Paci, Graduate Research Fellow, Nanomedicine, Houston Methodist Research Institute, TX
3. Vincenzo Di Bello, Visiting Graduate Fellow, Nanomedicine, Houston Methodist Research Institute, TX
4. Lavanya Saxena, PhD candidate, Bioengineering, Rice University, TX
5. Laura Segatori, Professor, Bioengineering, Rice University, TX
6. Corrine Ying Xuan Chua, Assistant Professor, Nanomedicine, Houston Methodist Research Institute, TX
7. Alessandro Grattoni, Professor, Nanomedicine, Houston Methodist Research Institute, TX

Keywords: Electrogenetic control, cell therapy, electrical stimulation, bioelectronics, control system

Abstract

Biohybrid cell therapy platforms require the seamless integration of electrogenetic cell interfaces with bioelectronic systems. While electrogenetic cells can be synthetically engineered to modulate ion channel activity and gene expression in response to potassium chloride (KCl) stimulation, their electrical responsiveness remains insufficiently characterized and lacks reproducible validation. This study investigates the systems-level behavior of electrogenetic Human Embryonic Kidney (eHEK) cells, conceptualized as stimulus-responsive biological control units governed by a black-box genetic circuit. While the NFAT signaling pathway involved in cellular responses is well characterized, our hypothesis is that treating the genetic circuit as a black box may enable functional abstraction at the systems level. To establish a cellular control system, voltage-gated calcium channels were ectopically expressed to facilitate membrane depolarization in response to KCl stimulation or electrical pulses. This depolarization induced calcium influx, activating the calmodulin–calcineurin signaling pathway, which led to NFAT dephosphorylation, nuclear translocation, and subsequent activation of a target promoter driving transgenic protein expression. Secreted Embryonic Alkaline Phosphatase (SEAP) levels were quantified in response to stepwise and continuous KCl dosing, as well as pulsatile electrical stimulation. SEAP production increased with both dose and duration of KCl exposure, up to thresholds of 70mM concentration and 7 days respectively, beyond which overstimulation led to reduced cell viability. Electrically, eHEK cells responded to biphasic pulse stimulation at 22.5mA current amplitude, 4ms pulse width, and 10 Hz frequency,

producing maximal SEAP levels over 3 days with short-duration pulse trains. These findings will inform the development of electrical stimulation protocol and optimized schedule for eHEK cells, tailored to the kinetics of protein release and desired expression output levels. Further modeling of the input-output dynamics of electrogenetically controlled cells will support the electronic design of biohybrid cell therapy devices.

#28

Diagnostic Nanomedicine: Tracking Natural Killer (NK) Cell Adoptive Immunotherapy in Pancreatic Cancer Using layer by layer(LbL)-Coated Perfluorocarbon Nanodroplets(PFC-NDs)

Ali Haghighat Mesbahi, Dmitry Nevozhay, James A. Bankson, Konstantin V. Sokolov
Department of Imaging Physics, The UT M.D. Anderson Cancer Center

Keywords: Diagnostic Nanomedicine, PFC-NDs; LbL Coating; NK Cell Immunotherapy; ^{19}F MRI Tracking

Introduction:

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies, characterized by late diagnosis, limited responsiveness to conventional therapies, and an immunologically “cold” tumor microenvironment[1]. Adoptive NK cell immunotherapy has emerged as a promising treatment, offering tumor-targeting activity independent of prior antigen recognition[2]. However, its clinical efficacy in PDAC is hindered by poor NK cell persistence, limited tumor infiltration, and a lack of reliable methods to monitor NK cell biodistribution and therapeutic activity in vivo. To address these challenges, we developed a diagnostic nanomedicine platform using LbL dextran-coated PFC-NDs for efficient NK cell labeling and noninvasive monitoring via quantitative ^{19}F magnetic resonance imaging (MRI). This approach integrates diagnostic imaging with immune modulation to improve NK cell tracking, viability, and tumor-targeting capability, ultimately aiming to enhance the therapeutic outcomes of NK cell–based immunotherapy in PDAC.

Hypothesis:

We hypothesize that optimized LbL dextran-coated PFC-NDs can enhance the labeling efficiency, viability, and functional uptake of adoptively transferred natural NK cells. By providing a biocompatible and stable platform for intracellular delivery, these NDs are expected to preserve NK cell activity while allowing for sensitive and quantitative tracking using ^19F MRI.

Methods:

PFC-NDs were coated with a single layer of dextran sulfate (DX) using Layer-by-Layer (LbL) deposition in four different buffer systems, varying DX concentrations and molecular weights to optimize coating thickness and uniformity. The coated PFC-NDs were systematically evaluated for uniformity (hydrodynamic diameter), stability (cell incubation), and NK cell uptake (labeling efficiency). Labeling efficiency was determined by assessing both NK cell uptake and cell viability. Furthermore, the sensitivity of ^19F MRI was determined by measuring signal intensity from labeled NK cells, establishing detection thresholds relevant for future in vivo monitoring applications.

Results: Single-layer DX LbL coating in HEPES buffer with 13 kDa DX at a 1:1 weight ratio to PFC-NDs yielded uniform, stable nanodroplets that preserved maximal cell viability and significantly enhanced NK cell uptake. Labeled NK cells retained high viability and functional activity, confirming suitability for adoptive transfer. Quantitative ^19F MRI demonstrated robust and reproducible signals at clinically relevant cell numbers, establishing the feasibility of this platform for real-time, noninvasive monitoring of NK cell therapies in PDAC.

Conclusion(s):

Optimized one-layer DX LbL coating can effectively label NK cells while preserving viability and enabling sensitive ^{19}F MRI–based monitoring. This dignostic platform offers both diagnostic capabilities and potential to guide NK cell–based immunotherapy, representing a promising strategy to improve therapeutic outcomes and real-time monitoring in pancreatic cancer.

Acknowledgements

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#29

Engineering pH-Responsive Dendrimer–STAT3 Inhibitor Conjugates for Intracellular Delivery

Chenikkayala Siva Sankara,[1] Akanksha Ramadas Shanbhag,[1] Daniel Rincón Díaz,[1] Caitlyn A. Hodges,[2] Chenglong Li,[3] Jeffrey K. Harrison,[2] Fan Zhang*[1,2,4]

[1]Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville 32610

[2]Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville 32610

[3]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville 32610

[4]Department of Chemical Engineering, University of Florida, Gainesville 32610

KEYWORDS: Dendrimer nanoconjugates, STAT3 inhibition, drug delivery, linker chemistry, controlled release

INTRODUCTION: Signal transducer and activator of transcription 3 (STAT3) is persistently activated in myeloid-derived suppressor cells (MDSCs), promotes tumor-associated immunosuppression and poor clinical outcomes in over 70% of cancers. Despite decades of research, no FDA-approved small-molecule STAT3 inhibitor is available. Although LLL12 is a potent STAT3 inhibitor that prevents phosphorylation/dimerization, and activation,[1] its poor solubility, low bioavailability, rapid clearance, and toxicities hinder clinical translation. These limitations highlight the need for improved delivery strategies to fully realize the therapeutic potential of STAT3 inhibitors.

HYPOTHESIS: Our recent study show that Generation-6 hydroxyl-terminated PAMAM-dendrimers selectively target myeloid cells in tumors and lymphoid organs due to their ultra-small size and neutral surface.[2] Leveraging this property, we propose pH-sensitive dendrimer–LLL12 conjugates will improve pharmacological properties and selectively deliver STAT3 inhibitors to MDSCs in the tumor microenvironment, enhance therapeutic efficacy and tolerability in cancer.

METHODS: LLL12 conjugated to G6-hydroxyl-terminated PAMAM-dendrimers via pH-responsive carbamate, amide, or hydrazone linkers. Purified conjugates were characterized by NMR and HPLC; size and ζ-potential were measured by DLS. Aqueous solubility of dendrimer–LLL12 conjugates was measured at LLL12-equivalent concentrations. Stability and pH-triggered release were evaluated at physiological (pH 7.4) and intracellular (pH 4.5) conditions. *In vitro* potency and cytotoxicity were assessed in THP-$1_{STAT3\text{-}luc}$ cells. Immunomodulatory effects were tested in primary mouse bone-marrow cultures by monitoring monocytic-MDSC expansion and antigen-presenting cell (APC) maturation. Toxicity profile of hydrazone-linked G6–LLL12 (G6–LLL12H) and free LLL12 was evaluated in healthy mice. Efficacy was evaluated in KR158 tumor-bearing mice.

RESULTS: Three G6–LLL12 conjugates were synthesized in excellent yields (91–95%) by covalently attaching the STAT3 inhibitor LLL12 to G6-OH PAMAM-dendrimers through carbamate, amide, and hydrazone linkers. Notably conjugation improved LLL12 aqueous solubility (up to 10 mg/mL) and reduced cytotoxicity, while maintaining dendrimer size and surface charge. All conjugates exhibited high stability at physiological pH but released drug in a sustained, pH-dependent manner under acidic conditions, with release rates following the order: hydrazone > amide > carbamate. Release kinetics strongly correlated with *in vitro* potency and toxicity. Among them, the G6–LLL12H displayed greatest potency (IC50 = 0.42 ± 0.035 μg/mL), comparable to free LLL12 and superior to other conjugates. In primary bone marrow cultures G6–LLL12H effectively suppressed monocytic-MDSC expansion and promoted APC maturation.[3] Systemic G6–LLL12H administration preserved hepatocyte structure with reduced toxicity and no inflammation, whereas LLL12 induced hepatic toxicity and resulted in significant weight loss (***$p<0.001$). The G6-LLL12H selectivity was observed with increased survival of tumor-bearing mice than free LLL12. These results demonstrate the critical role of linker chemistry in determining release, potency, and immunomodulation, establishing G6–LLL12H as a promising pH-responsive delivery system.

CONCLUSIONS: Rationally designed pH-sensitive dendrimer–LLL12 conjugates overcome key pharmacological limitations of STAT3 inhibitors. G6–LLL12H achieved controlled release with optimal solubility, stability, and immunomodulatory activity against M-MDSCs. These findings highlight their potential as a targeted platform for STAT3 modulation in cancer therapy, with ongoing *in vivo* studies evaluating therapeutic efficacy, and synergy with other anticancer treatments.

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#30

Nanoscale TRAIL Liposomes Customized to Target Specific Colorectal Cancer Mutation States

Ehsan Aalaei[1], Zhenjiang Zhang[1], Benjamin Kaufman[1], Xiling Shen[2], Michael R. King[1]
[1]Department of Bioengineering, Rice University, Houston, TX, USA
[2]GI Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Introduction: Colorectal cancer (CRC) commonly spreads to the liver, and even after surgical removal of liver metastases, 50–75% of patients experience recurrence within two years [1]. Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) can selectively trigger apoptosis in cancer cells, but its clinical use has been limited by insufficient activity against resistant tumor types. The King Lab developed phase-separated nanoscale liposomes designed to cluster TRAIL at high local density, thereby enhancing activation of death receptors (DR4/DR5) and selectively triggering apoptosis in cancer cells. These TRAIL-conjugated liposomes attach to circulating immune cells, extending exposure and increasing the cancer cells' encounter rate. Physiologic shear potentiates death-receptor signaling through synergistic mechanotransduction via fluid shear stress (FSS), which activates the Piezo1 channel and results in extracellular calcium influx, thereby amplifying the apoptosis pathway in cancer cells [2].

Hypothesis: By tuning the TRAIL-to-liposome ratio and membrane composition, these particles can be tailored to match the receptor distribution of different CRC genotypes, including resistant subtypes, to create a neoadjuvant therapy to reduce post-surgical recurrence.

Methods: CRC cell lines DLD-1, SNU-C2B, RKO, and HT29 were used to compare soluble TRAIL with TRAIL-conjugated liposomes. Phase-separated nanoscale liposomes were prepared from DSPC:DOPC at a 3:1 molar ratio. TRAIL proteins were covalently conjugated at defined TRAIL:liposome loadings of 25:1, 50:1, 100:1, 200:1, and 400:1 to modulate ligand density and nanoscale presentation. Phase separation was verified on composition-matched giant unilamellar vesicles (GUVs) using confocal microscopy (Figure 1-b), and the size distributions of naked versus TRAIL-conjugated liposomes were quantified (Figure 1-c). Increasing TRAIL loading resulted in larger mean diameters, consistent with successful surface association. Cells were treated with either free TRAIL or one of the TRAIL-liposome formulations. Apoptosis was quantified 24 h post-treatment using Annexin V/propidium iodide staining followed by flow cytometry, and data were summarized as percent apoptotic cells and percent viability for each. Formulations differed in TRAIL loading and membrane composition to evaluate a mutation-tailored design across CRC genotypes, enabling comparison of responses in TRAIL-sensitive versus TRAIL-resistant lines under identical dosing and assay conditions.

Results: The results show that free TRAIL significantly reduced viability in DLD-1 and SNU-C2B cells, consistent with their known sensitivity. Across these lines, all TRAIL-liposome formulations produced comparable or slightly improved reductions in viability, indicating that liposomal conjugation maintains TRAIL's apoptotic function (Figure 1). In contrast, RKO and HT29 exhibited minimal response to free TRAIL but showed marked reductions in viability with liposomal TRAIL formulations; most notably, F-50 for RKO and F-100 for HT29 (Figure 1). These results suggest that liposomal presentation of TRAIL can enhance receptor engagement and apoptotic signaling in resistant cells.

Conclusion: The findings support our hypothesis that TRAIL-liposome formulations can be tuned to address mutation-specific resistance. The differential responses of RKO and HT29 highlight the importance of optimizing TRAIL loading and liposome design based on the genetic context of the tumor. The data justify continued development of mutation-specific formulations, with future work focused on optimizing TRAIL concentrations, refining liposome structure and TRAIL density, and evaluating efficacy in animal models.

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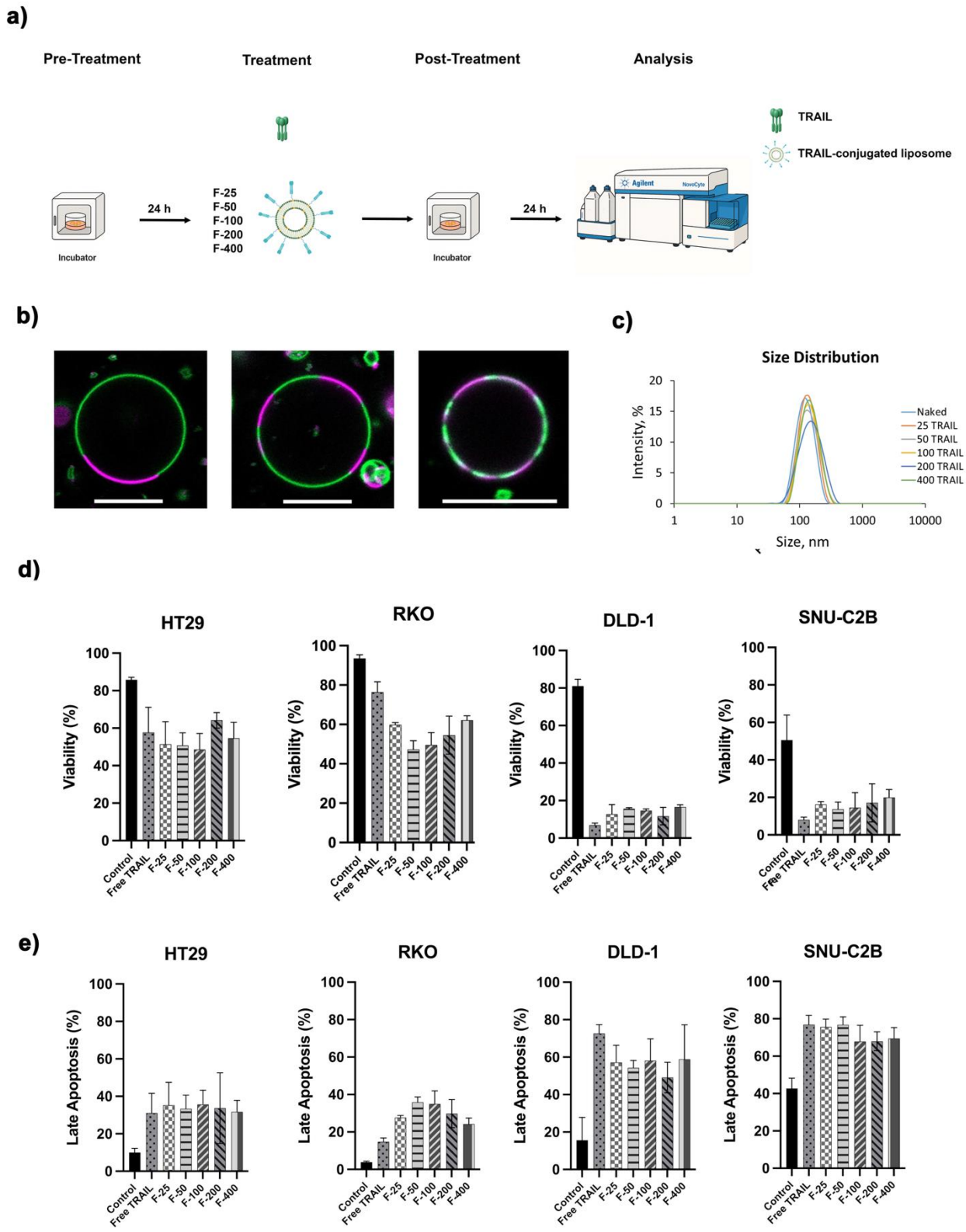

Figure 1: a) Workflow. b) Confocal microscopy images of GUVs showing separation of phases (green = DSPC, red = DOPC) to different extents. The GUVs are composed of the same lipid bilayer membrane structure as nanoscale liposomes. Scale bars = 10 µm [3]. c) Size distribution of naked liposomes and liposomes conjugated with varying numbers of TRAIL molecules. d) Cell viability measured 24 hours after treatment. e) TRAIL-induced apoptosis in four colorectal cancer cell lines after 24 hours of treatment. Error bars represent standard deviations from replicate experiments.

#31

Intratumoral Biodegradable Nanofluidic Platform for Localized Multimodal Immunotherapy Enhances Tumor Eradication and Immune Memory

Authors: Jingyi Wang[1,2], Francesco Manfredi[1,3], Eleonora Molinari[1,4], Casey Lewis[1], Madison Alexandra Deeson[1], Danilo Settis[1], Sally Peng[1], Robin Vander pol[1], Marzia Conte[4], Corrine Ying Xuan Chua[1], Alessandro Grattoni[1,5,6]

1. Nanomedicine Department, Houston Methodist Research Institute
2. Department of Nuclear Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine
3. College of Engineering, Swansea University, Swansea
4. Department of Mechanical and Aerospace Engineering, Politecnico di Torino
5. Department of Surgery, Houston Methodist Hospital
6. Department of Radiation Oncology, Houston Methodist Hospital

Keywords: Biodegradable nanofluidic platform, drug delivery, immunotherapy, tumor immune landscape, immune memory

Introduction: Tumor-infiltrating lymphocytes (TILs) are essential players in the tumor immune microenvironment (TIME), often correlating with favorable prognosis in multiple cancers. Although immunotherapy has emerged as a transformative cancer treatment, its efficacy is frequently hampered by immune evasion and toxicity associated with systemic delivery. Multi-drug regimens targeting complementary immune pathways offer promise but are limited by dose-limiting systemic toxicity.

Hypothesis: We hypothesize that localized, sustained delivery of multiple immunotherapeutics via a biodegradable nanofluidic drug-eluting seed (b-NDES) can enhance anti-tumor efficacy, induce immune memory, and minimize systemic toxicity.

Methods: We engineered an implantable biodegradable nanofluidic platform (b-NDES) for intratumoral delivery of α-CTLA4, STING agonist, resiquimod (TLR7/8 agonist), IL-12, and α-CD40. In vivo efficacy was tested in murine models of triple-negative breast cancer (4T1), pancreatic cancer (KPC), and lung cancer (KLN205). Three- to five-drug combinations were evaluated for tumor regression, systemic immune responses, and toxicity. Immune memory was assessed by tumor rechallenge and IFN-γ secretion assays. Cytokine and chemokine responses were profiled via Olink proteomics, and the tumor immune landscape was characterized using CyTOF and imaging mass cytometry (IMC).

Results: The five-drug b-NDES regimen demonstrated superior efficacy, achieving complete tumor clearance in 3 of 6 mice in the 4T1 model and showing consistent antitumor activity across KPC and KLN205 models. In rechallenge experiments, previously cured mice rejected subsequent tumor inoculation and exhibited robust IFN-γ production from tumor-specific T cells, confirming durable immune memory. In the KPC abscopal model, b-NDES triggered systemic immune activation, leading to regression of untreated tumors. Cytokine profiling revealed a strong pro-inflammatory response in the five-drug group. Importantly, unlike systemic intraperitoneal administration, b-NDES delivery minimized toxicity, preserving body weight and temperature and avoiding liver damage.

Conclusion: Our findings highlight the b-NDES platform as a powerful tool for localized, multimodal immunotherapy. The sustained intratumoral delivery of synergistic agents enabled complete tumor eradication, systemic immune responses, and long-lasting memory without inducing the toxic effects commonly seen with systemic treatment. This approach offers a promising direction for future cancer immunotherapy strategies targeting aggressive, treatment-resistant tumors.

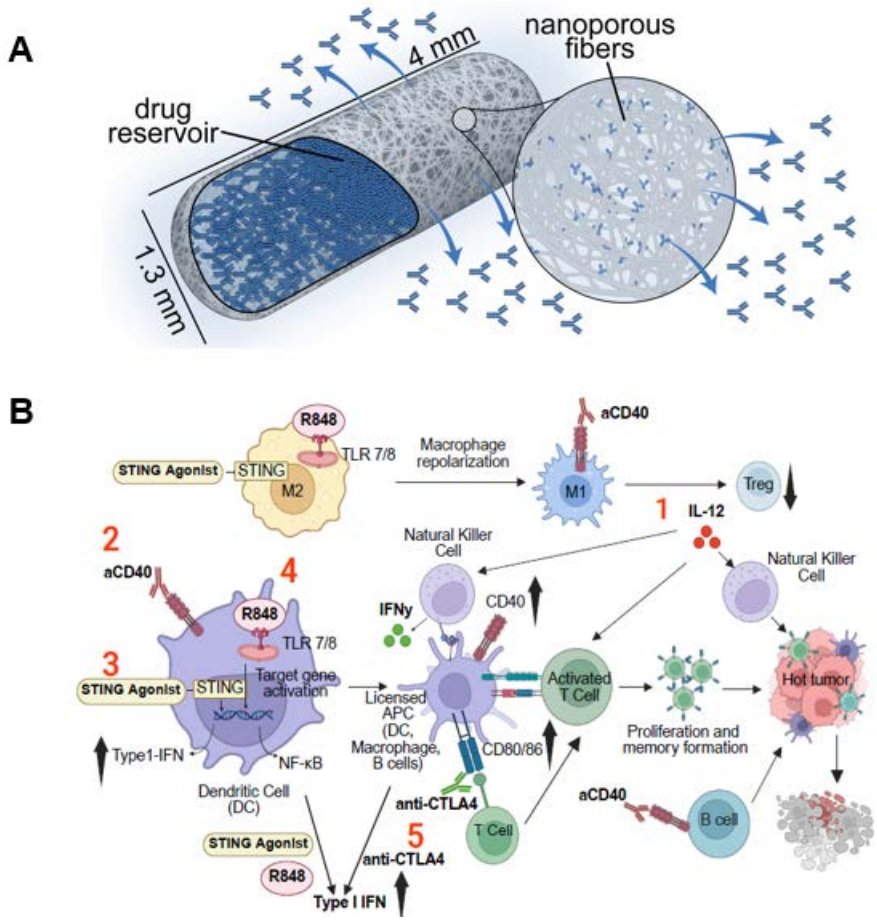

Figure 1. Biodegradable nanofluidic drug-eluting seed (b-NDES) platform and mechanisms of multimodal immunotherapy. (A) Schematic of the b-NDES implant showing drug reservoir and nanoporous fibers enabling sustained intratumoral release of immunotherapeutics. (B) Mechanistic illustration of the five-drug regimen.

#32

NanoLymph: A Nanotechnology-Based Device to Create a Tolerogenic Environment for Cell Transplantation

Danilo Settis[1,2], Nikitha Kota[1,3], Martina Concato[1,2], Liu Yongbin[1], Casey Lewis[1], Noemi Risso[1,2], Corrine Ying Xuan Chua[1], Alessandro Grattoni[1,4,5]

[1] Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
[2] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy
[3] Department of Biosciences, Rice University, Houston, TX, USA
[4] Department of Surgery, Houston Methodist Hospital, Houston, TX, USA
[5] Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX, USA

Keywords: Transplantation, Cell Therapy, Regulatory T cells, Implantable Device

Introduction
Cell therapies have increasing potential in treating various diseases by replacing or repairing damaged tissues. However, immune-mediated rejection remains a critical challenge to successful transplantation. Current strategies rely mostly on systemic immunosuppressive treatments, which have severe side effects and cause immune dysfunction.

Hypothesis
Our device harnesses a nanoporous membrane to release chemokines that recruit and activate regulatory T cells (Tregs). These Tregs play an important role in maintaining a tolerogenic environment which promotes cell engraftment.

Materials and Methods
NanoLymph is a nanotechnology-based device composed of 3D-printed biocompatible resin, a nylon mesh, a collagen scaffold, and a PES 30nm membrane. It contains a separate drug reservoir which releases CCL22, IL-2/JES6, MR1, and αCD8. It was tested in a mouse model with allogenic Leydig (TM3 cell line) cell transplantation, in which it promoted Treg activation and created a tolerogenic environment. Drug release, biodistribution, histology, and flow cytometry studies were used to evaluate its efficacy.

Results
The NanoLymph demonstrated integration with host subcutaneous tissue and increased cellular infiltration, vascularization, and lymphangiogenesis. The NanoLymph facilitated sustained release of chemokines which recruited $CD4^+CD25^+Foxp3^+$ Tregs, creating an immunomodulatory niche conducive to transplantation. In an MHC-mismatched transplant model, NanoLymph significantly prolonged the viability of allogeneic TM3 cells compared to systemic immunosuppression alone. Flow cytometry confirmed increased Treg:CD8 ratio within the device.

Conclusions
These findings demonstrate that NanoLymph effectively creates a tolerogenic environment that supports the survival of transplanted TM3 cells. As demonstrated by drug release and biodistribution analysis, the device releases chemokines locally. These chemokines recruit Tregs and deplete T effector cells, promoting immune tolerance for cell engraftment. These results establish NanoLymph as a promising alternative to conventional immunosuppressive therapies, offering a more efficient and sustainable solution for cell transplantation.

Acknowledgments:
This work is supported by Houston Methodist Research Institute (A.G., C.Y.X.C.)

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#33

Tuberculosis Biosensors: Polymer-Based Resistive Biosensors for Rapid Detection of *Mycobacterium tuberculosis*

Vincenzo Di Bello[1 2], Jaskirat Singh Batra[1], Chinnaswamy Jagannath, Edward Graviss, Corrine Ying Xuan Chua[1,3,5], Alessandro Grattoni[1,3,5*]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA;

[2]Dipartimento di Ingegneria Chimica dei Materiali e della Produzione Industriale, Università degli studi di Napoli "Federico II, Piazzale Tecchio 80, 80125 Napoli, Italy;

[3]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA;

*Correspondence: agrattoni@houstonmethodist.org (A.G.); Tel.: +1-(713)-441-7324 (A.G.)

Keywords
Tuberculosis; Biosensor; Molecularly Imprinted Polymer; PEDOT:PSS; Mycolic Acid

Introduction
Tuberculosis (TB) remains one of the world's deadliest infectious diseases, current diagnostic methods for its detection rely on blood cultures and require several days for the results. A rapid, non-invasive approach based on saliva analysis could substantially improve early detection and patient management. Mycolic acids, long-chain fatty acids unique to the *Mycobacterium tuberculosis* cell wall, are present in detectable concentrations in the respiratory tract and constitute promising molecular biomarkers.

Hypothesis
We hypothesize that a biosensor integrating molecularly imprinted polymer (MIP) fabrication with a conductive PEDOT:PSS matrix that can selectively recognize mycolic acid and enable rapid, label-free, electrical detection suitable for both benchtop and handheld diagnostic platforms.

Methods
PEDOT:PSS was chosen as the conductive polymer matrix due to its stability, ease of manufacturing, and tremendous potential in biosensing applications. Molecularly imprinted polymers (MIPs) were fabricated by incorporating mycolic acid during polymer preparation, thereby creating selective recognition cavities upon template removal with chloroform. Non-imprinted polymers (NIPs), prepared under identical conditions without the template, were used as controls.

The protocol was refined to approximate a real-life situation and to better mimic physiological conditions. Specifically, mycolic acid was introduced in a water/Tween micellar solution, enabling testing under more biologically relevant conditions. In addition, electrode configuration was implemented by directly attaching copper pads and silver epoxy contacts to the polymer surface, establishing a robust two-wire system. This configuration reduced signal fluctuations and provided more stable readings over repeated cycles.

Results

MIP samples consistently exhibited lower baseline electrical resistance compared to NIPs after chloroform washing, confirming imprinting effects. Larger sample geometries, such as rectangles and large squares, produced more uniform measurements, whereas smaller geometries suffered from higher variability. A serial dilution enabled detection down to 100 ng/mL mycolic acid, although variability from surface distribution of mycolic acid on filter paper affected measurement precision. Transitioning from a four-probe to a two-probe copper–silver epoxy configuration provided a more direct readout of internal changes within the polymer matrix, minimizing surface artifacts and enhancing sensitivity. The implementation of aqueous detection mimicked a realistic condition, suggesting that the detection mechanism is still functional beyond organic solvents. Preliminary calibration curves showed concentration-dependent resistance changes, and ongoing optimization with wet-sensor electrochemical approaches indicates potential to further enhance sensitivity and reduce detection time (<1 min).

Conclusions

This work demonstrates the feasibility of using MIP-modified PEDOT:PSS sensors for rapid and selective mycolic acid detection as a surrogate marker for TB. While further optimization is required to improve sensitivity and reproducibility, the platform shows preliminary results for translation into handheld biosensors capable of non-invasive TB screening. Future efforts will focus on refining sensor architecture, validating in clinically relevant aerosol samples, and benchmarking performance against established diagnostic techniques. Overall, these results highlight the translational potential of this point-of-care TB biosensor.

#34

Engineering a Subcutaneous Artificial Lymph Node for Antigen-Specific Immune Modulation in Type I Diabetes

Madison A. Deeson[1,2], **Danilo Settis[1]**, Nikitha Kota[1,3], Casey Lewis[1], Dia Gupta[1,3], Yongbin Liu[1], Jingyi Wang[1], Corrine Ying Xuan Chua[1], Alessandro Grattoni[1,5,6]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA
[2]Texas A&M University Naresh K. Vashisht College of Medicine, Bryan, TX 77807, USA
[3]Department of Biosciences, Rice University, Houston, TX, USA
[4]Department of Surgery, Houston Methodist Hospital, Houston, TX 77030, USA
[5]Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX 77030, USA

Keywords: biomaterials, implantable devices, immunotherapy, tolerogenic dendritic cells, regulatory T cells, autoimmunity, Type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an autoimmune disease in which pancreatic β cells are progressively destroyed by autoreactive T cells, ultimately leaving patients dependent on lifelong insulin replacement. Current prevention strategies are limited by systemic immunosuppression, which carries significant risks of infection and malignancy. To address this, we developed the NanoLymph, a subcutaneous artificial lymph node engineered to locally modulate the immune system and halt the progression of T1D without systemic immunosuppression.

Hypothesis

We hypothesize that the NanoLymph can induce antigen-specific tolerance to the islet autoantigen glutamic acid decarboxylase 65 (GAD65) by recruiting dendritic cells, reprogramming them toward a tolerogenic phenotype in the presence of GAD65, and promoting the differentiation of naïve $CD4^+$ T cells into protective GAD65-specific regulatory T cells. These $GAD65^+$ Tregs can then migrate to the pancreas to preserve β cell function in T1D.

Methods

In vitro studies evaluated whether dendritic cells treated with calcitriol and dexamethasone adopt a tolerogenic phenotype ($CD80^{low}CD86^{low}PD\text{-}1^+$) and, upon maturation with an MHC II–restricted epitope of ovalbumin ($OVA_{323–339}$), drive the generation of antigen-specific regulatory T cells in dendritic cell–T cell co-cultures. Future work will assess whether the NanoLymph can reproduce this biology in vivo by inducing GAD65-specific tolerance in nonobese diabetic (NOD) mice.

Results

In vitro, dendritic cells treated with calcitriol and dexamethasone acquired a tolerogenic phenotype and effectively promoted the differentiation of OVA-specific regulatory T cells,

demonstrating the feasibility of our therapeutic regiment for antigen-specific immune modulation.

Conclusions

Our therapeutic regiment successfully generates antigen-specific regulatory T cells in vitro, establishing a foundation for future in vivo validation. If translated effectively, this platform could transform the treatment of T1D and other autoimmune diseases by inducing targeted immune tolerance while avoiding the risks of systemic immunosuppression.

Acknowledgements

This work was supported by the Houston Methodist Research Institute (A.G., C.Y.X.C.) and by the Breakthrough Type 1 Diabetes Innovative Award [Grant No. 1-INO-2025-1712-A-N].

#35

Assessment of Targeted Gold Nanorods as Photoacoustic Imaging Contrast Agents

Farah M. Al Hadad[1,2], Antonio Martino PhD[1], Kara Hood PhD[3], Blake C. Fallon BA[1], Nilesh Mathuria MD[4], Richard C. Willson PhD[5], Richard R. Bouchard PhD[6,7], Carly S. Filgueira, PhD[1,8]

1 Department of Nanomedicine, Houston Methodist Research Institute; Houston, TX

2 Department of Biomedical Engineering, Cullen College of Engineering, University of Houston; Houston, TX

3 Infectious Disease, Houston Methodist Academic Institute; Houston, TX

4 Houston Methodist Heart and Vascular Center, Houston Methodist Research Institute, Houston Methodist Hospital; Houston, TX

5 Department of Chemical and Biomolecular Engineering, University of Houston; Houston, TX

6 Department of Imaging Physics, The University of Texas MD Anderson Cancer Center; Houston, TX

7 MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences; Houston, TX

8 Department of Cardiovascular Surgery, Houston Methodist Research Institute; Houston, TX

Keywords: Photoacoustic-Ultrasound imaging, metallic nanoparticles, surface functionalization, targeted delivery, nano Differential Scanning Fluorimetry

Introduction: Current imaging modalities (such as fluoroscopy, magnetic resonance imaging, and computed tomography) used by clinicians lack the ability to differentiate between non-conducting and electrically conducting cardiomyocytes, comprising the cardiac conduction system (CCS). Identifying the CCS intraoperatively would be advantageous for reducing repeat procedures and confirming success. Photoacoustic imaging (PAI) offers an imaging platform for real-time, high-resolution, low-noise tissue visualization through pulsed light absorption and ultrasonic signal detection.[1] Gold nanorods (GNRs) are a promising PAI contrast agent due to their tunable plasmonic properties. To differentiate electrically conducting cardiomyocytes, an anti-contactin 2 (Cntn2)[2] antibody can be conjugated to GNRs for targeted, contrast-enhanced PAI.

Hypothesis: We hypothesize that GNRs functionalized with anti-Cntn2 can serve as strong, targeted PAI contrast agents for precise imaging of the CCS.

Methods: To quantify PAI signal intensity of GNRs, a tissue-mimicking phantom was created using a custom 3D printed silicone mold, filled with 0, 27, 54, 108, 216, and 432 mg/L of GNRs in 8% gelatin, and imaged with PAI. Carboxyl pegylated GNRs (55 x 15 nm, Nanocomposix) were conjugated with either anti-Cntn2 or IgG (non-specific control Ab) using EDC/sulfoNHS chemistry for 15 minutes in a reaction buffer of 0.1 M MES (pH= 6.00) followed by a 1 hour antibody incubation in a reaction buffer of 1x PBS (pH= 7.4). Nano differential scanning fluorimetry (nanoDSF)[3] was used to confirm successful antibody conjugation without inducing structural changes, assessed by thermal unfolding (changes in melting temperature, Tm). In vitro cell culture studies using U87MG cells expressing Cntn2 were performed to validate specific cell recognition of the Cntn2 pegylated GNRs at varying concentrations after a 2 hr incubation. Gold content was measured using inductively coupled plasma – optical emission spectroscopy (ICP-OES).

Results: PAI data of the phantoms showed a linear relationship between GNR concentration and PAI signal at 780 and 800 nm (R^2 = 0.9863, 0.9898; respectively), confirming that GNRs can be used as concentration-dependent PAI contrast agents. nanoDSF confirmed stable protein conformation after conjugation demonstrated by similar melting temperatures for IgG alone (Tm=69.70 ± 0.34°C) and IgG-PEG-GNRs (Tm= 69.06 ± 0.90°C). ICP-OES revealed a significant increase Cntn2-PEG-GNRs binding to U87MG cells (****p< 0.0001) compared to PEG-GNRs and IgG-PEG-GNRs.

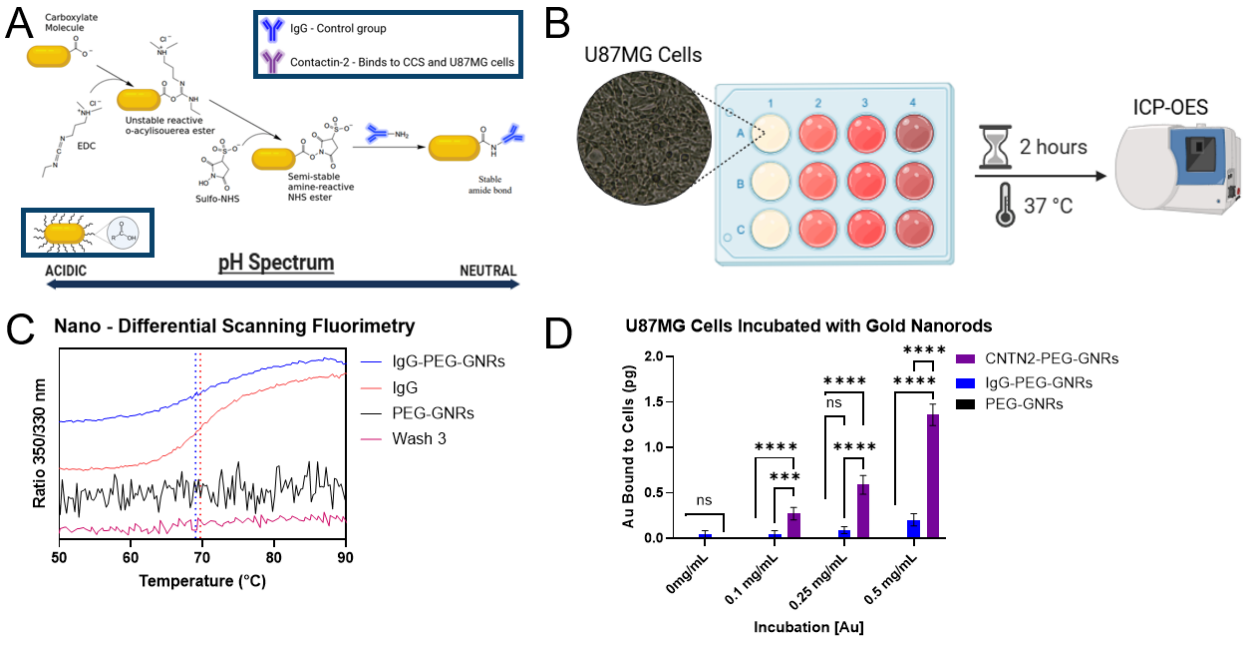

Figure 1. Schematic of A) GNP Ab conjugation chemistry and B) cell studies. C) Nano Differential Scanning Fluorimetry (offset for clarity) ratio 350/330 nm for IgG-PEG-GNRs and

PEG-GNRs. D) ICP-OES measurements of gold content in cells incubated with PEG-GNRs, IgG-PEG-GNRs, or Cntn2-PEG-GNRs.

Conclusion: GNRs were proven as strong contrast agents demonstrated by their high and dose-dependent PAI signal. Further, targeted cell binding was achieved by modifying their surface chemistry to include specific antibodies, confirmed by the greater presence of gold in cells expressing the complementary antigen to anti-Cntn2. To our knowledge, we also show for the first time use of nanoDSF with metal nanoparticles to confirm successful antibody conjugation with preserved bioactivity. Overall, this work would provide clinicians with real time visualization of the CCS improving procedural success.

Acknowledgements: This work was supported by the John S. Dunn Foundation Collaborative Research Award, and Dr. Samuel John supported the PAI data acquisition at MD Anderson Cancer Center.

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#36

Intratumoral Biodegradable Nanofluidic Seed Platform Improves Tumor-Specific Delivery of CD47 Antibody in Breast Cancer

*Jingyi Wang, PhD[1,3], Eleonora Molinari, MS[1,2], Francesco Manfredi, MS[1,2], **Casey Lewis**, BA[1], Corrine Ying Xuan Chua, PhD[1], Alessandro Grattoni, PhD[1,4,5].*

[1]Nanomedicine Department, Houston Methodist Research Institute, Houston, TX 77030, USA.
[2]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino 10129, Italy
[3]Department of Nuclear Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[4]Department of Surgery, Houston Methodist Research Institute, Houston, TX 77030, USA.
[5]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA.

CD47 is a transmembrane protein that acts as a "don't eat me" signal to macrophages and other immune cells, inhibiting cancer cell phagocytosis. CD47-targeting antibodies have emerged as promising cancer therapies by blocking CD47-SIRPα interaction, thereby promoting immune-mediated clearance of cancer cells. However, clinical applications face challenges due to widespread CD47 expression on normal cells, leading to off-target effects and hematologic toxicities, including anemia from red blood cell binding. We developed a biodegradable nanofluidic drug-eluting seed (b-NDES) platform for controlled and sustained intratumoral release of CD47 antibodies. To compare biodistribution and retention, we used fluorescently labeled CD47 monoclonal antibody (CD47-AF647) delivered via three methods: b-NDES, intraperitoneal (IP), and intratumoral (IT) injections in a 4T1 breast cancer murine model. In vivo fluorescence imaging was conducted to monitor real-time drug biodistribution, followed by ex vivo analysis of tumors and major organs. In vivo and ex vivo fluorescence imaging confirmed that b-NDES enabled continuous release of CD47 antibodies for up to one month, resulting in enhanced tumor retention and minimized systemic exposure, compared to IP and IT injections. Body weight and temperature analysis indicated reduced systemic toxicity in the b-NDES group, with no significant fluctuations between the treatment and control groups. These results demonstrate that the b-NDES platform offers a promising approach for sustained tumor-specific delivery with minimized systemic side effects, enhancing the therapeutic index of CD47-targeted therapies.

Acknowledgements

Funding support was received from Nancy Owens Breast Cancer Foundation and the Houston Methodist Research Institute.

Conflicts of Interest

Alessandro Grattoni is an inventor of intellectual property licensed by Continuity Biosciences. The other authors declare no conflict of interest.

#37

Optimizing NICHE Pre-Vascularization and Systemic Immunosuppression for Islet Engraftment in Non-Human Primate Models

Arya Venugopalan[1], Jocelyn Nikita Campa-Carranza, PhD[1,2], Melissa A. Willman, Gabrielle E. Rome[1], Alexander Rabassa[6], Rim Ouni, PhD[1], Elisa Balboni, MS[1,3], Henry Seaborne[1], Jesus Paez-Mayorga, MD, PhD[1], Corrine Ying Xuan Chua, PhD[1], Norma S Kenyon, PhD[6,7,8,9], and Alessandro Grattoni, PhD[1,4,5]

[1]Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2]Department of Medicine and Health Sciences, Tecnologico de Monterrey, NL, Mexico; [3]Department of Applied Science and Technology, Torino, Italy; [4]Department of Surgery, Houston Methodist Hospital, TX, USA; [5]Department of Radiation Oncology, Houston Methodist Hospital; [6]Diabetes Research Institute, University of Miami Miller School of Medicine, FL, USA. [7]Department of Biomedical Engineering, University of Miami, FL, USA. [8]Department of Surgery, University of Miami Miller School of Medicine. [9]Department of Microbiology and Immunology, University of Miami Miller School of Medicine.

Key Words: Islet Engraftment, Pre-Vascularization, NICHE, Immunosuppresion

Introduction

Successful transplantation of allogeneic islets relies on the establishment of a highly vascularized and immune-protected microenvironment to support cell engraftment and function. The NICHE (Neovascularized Implantable Cell Homing Encapsulation) device has demonstrated promise by combining local pro-angiogenic strategies with barriers to immune cell infiltration in murine models. However, optimal device pre-vascularization protocols for islet engraftment in non-human primate (NHP) models are yet to be defined.

Hypothesis

NICHE implantation with autologous Mesenchymal Stem Cells (MSC) will improve device pre-vascularization and support islet engraftment in NHPs.

Methods

To assess prevascularization, cynomolgus macaques (n=3) were subcutaneously implanted with four NICHE devices loaded with 625,000 autologous MSCs in Pluronic F127 hydrogel. One device per NHP was explanted weekly at 3-6 weeks and vessel density was quantified using a semi-automated Python script internally validated to quantify blood vessels on histology samples. To assess feasibility of islet engraftment, diabetic cynomolgus macaques (n=3) were subcutaneously implanted with NICHE-MSC devices. After 6 weeks of prevascularization, 6000 allogeneic IEQ/kg from mismatched donors were loaded into the NICHE devices. Induction immunosuppression with thymoglobulin was started 7 days pre-transplant, followed by maintenance anti-CD40L beginning on transplant day. Serial blood and tissue sampling, including device explants at post-transplant day (POD) 90 enabled assessment of vascular, immunologic and engraftment outcomes.

Results

Serial analysis of the NICHE devices revealed robust, time-dependent vascularization, with peak vascular density at 6 weeks post-implantation (4.59 ± 0.608 $1/mm^2$) Fibrotic encapsulation was

limited across timepoints, averaging 150–170 μm in thickness. A reduction in capillary area fraction and density was observed from week 3 to week 6, likely stemming from vascular remodeling and maturation. IHC analysis of POD 90 NICHE showed insulin-positive cells, demonstrating NICHE prevascularization was conducive to islet permanence and survival in the device. In addition, C-peptide levels became detectable in serum after transplant, further supporting islet graft function.

Conclusion
These findings establish that pre-vascularization using autologous MSCs is effective and enables durable islet engraftment in NHPs. This strategy illustrates an important step toward clinically translatable, islet transplantation using the NICHE platform.

Acknowledgements
This work is supported by grant 1R01DK133610-01 from the National Institutes of Health.

Competing Interests
CYXC, JPM, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare they have no competing interests.

#38

Immune Signatures of Localized Immunosuppressant Delivery in Islet Transplantation

Jocelyn Nikita Campa-Carranza[1,2], Simone Capuani[1], **Jesus Paez-Mayorga[1]**, Melissa A. Willman[3], Alexander Rabassa[3], Ashley L. Joubert[1], Tommaso Bo[1], Letizia Franco[1,4], Marzia Conte[1,4], Ana L. Anaya-García[1,2], Gabrielle E. Rome[1], Rim Ouni[1], Camden A. Caffey[1], Dora M. Berman[3], Junjun Zheng[5,6], Corrine Ying Xuan Chua[1], Shu Hsia Chen[5,6], Norma S. Kenyon[3,7,8,9,10], Alessandro Grattoni[1,11,12]

[1]Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2]School of Medicine and Health Sciences, Tecnologico de Monterrey, NL, Mexico; [3] Diabetes Research Institute, University of Miami, FL, USA; [4] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy; [5] Center for Immunotherapy Research, Houston Methodist Research Institute; [6] Immunomonitoring Core, Houston Methodist Research Institute; [7] Department of Surgery, Miller School of Medicine, University of Miami; [8] Department of Microbiology and Immunology, Miller School of Medicine, University of Miami; [9] Department of Biomedical Engineering, University of Miami; [10] Department of Biochemistry and Molecular Biology, University of Miami; [11] Department of Surgery, Houston Methodist Hospital, TX, USA; [12] Department of Radiation Oncology, Houston Methodist Hospital.

Introduction

Systemic immunosuppression remains the cornerstone for allogeneic transplant rejection prophylaxis, but its chronic use causes substantial toxicity. Conceptually, local, site-specific immunomodulation offers a promising alternative, yet comparative insight into how immunosuppressants behave when delivered directly to the graft is lacking.

Hypothesis

Local delivery of CTLA4-Ig, anti-lymphocyte serum (ALS), anti-CD40L, anti-CD2, and anti-IL-6 via the NICHE device elicits differential immune profiles at the graft site and systemically.

Methods

We leveraged the Neovascularized Implantable Cell Homing and Encapsulation (NICHE) device, a subcutaneous vascularized platform that enables co-delivery of pancreatic islets and immunosuppressive agents within a spatially defined environment. Using this system, we independently evaluated five clinically relevant agents delivered locally in immunocompetent rats. Safe and immunologically active dose ranges were defined in vitro using mixed lymphocyte reactions, perifusion glucose-stimulated insulin secretion, and islet viability assays. In vivo, we characterized drug pharmacokinetics, biodistribution, and immune signatures at the graft site, in circulation, and across systemic compartments in an allogeneic islet transplantation model.

Results

All agents achieved sustained release within the NICHE without impairing islet viability or function. Drug levels were highest in the local transplant microenvironment, with systemic concentrations up to 100-fold lower, demonstrating the capacity of this platform to minimize off-target exposure. Distinct immune profiles were observed for each agent: CTLA4-Ig broadly suppressed effector infiltration but also depleted Tregs; ALS induced lymphodepletion while preserving Tregs and promoting M2-like macrophage polarization; anti-CD40L reduced T cell

costimulation and antigen-presenting cell activity while sparing regulatory subsets; anti-CD2 partially suppressed T cells, preserved Tregs, and induced early anti-inflammatory macrophage polarization; and anti-IL-6 failed to suppress infiltration and instead enhanced cytotoxic activity.

Conclusion

This study establishes a comparative framework for localized immunomodulation in cell transplantation. Sustained, site-specific delivery preserved islet function while generating agent-specific immune profiles. By revealing how individual immunosuppressants shape the local immune landscape, this study provides the mechanistic foundation needed to design rational, multi-targeted regimens that could ultimately eliminate reliance on systemic immunosuppression in cell therapy.

Acknowledgements

This work was supported by Breakthrough T1D (BT1D 2-SRA-2022-1224-S-B, AG, NK), NIH NIDDK (R01 DK133610-01, AG, NK), and Houston Methodist Research Institute (AG). The authors thank Dr. David L. Haviland and Elizabeth Jardinella from the flow cytometry core of Houston Methodist Research Institute for their support during cytometer setup.

Key words

Localized drug delivery, Immunomodulation, Biodistribution, Islet transplantation, cell therapy

Competing Interests

SC, JPM, CYXC, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare they have no competing interests.

#39

Modulation of Local and Systemic Immune Responses to Allogenic Islet Transplants by Immunosuppressive Drug Combinations within a Subcutaneous Niche in Diabetic Rats

Rim Ouni, PhD[1], Nikita Campa-Carranza, PhD[1,2], Gabrielle Rome[1], Henry Seaborne[1], Elisa Balboni[1,3], Mia Girela[1,4], Sumita Dantu[1,4], Junjun Zheng, PhD [5,6], Shu Hsia Chen, PhD[5,6] Corrine Ying Xuan Chua, PhD[1], Alessandro Grattoni, PhD[1,7,8]

[1] Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2] Tecnologico de Monterrey School of Medicine, NL, Mexico; [3] Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy; [4]Wiess School of Natural Sciences, Rice University, TX, USA; [5] Center for Immunotherapy Research, Houston Methodist Research Institute; [6] Immunomonitoring Core, Houston Methodist Research Institute; [7] Department of Surgery, Houston Methodist Hospital, TX, USA; [8] Department of Radiation Oncology, Houston Methodist Hospital

Key words: Islet transplantation, NICHE, Drug Combinations, Immunomodulation

Introduction

Clinical Islet transplantation is an effective approach to restore glycemic control in patients with type 1 diabetes. However, its clinical application remains limited by the need for lifelong systemic immunosuppression, which is associated with significant risks and adverse effects. While combinatory immunosuppressive regimens have demonstrated enhanced efficacy in preventing graft rejection (Yue et al., 2024), systemic administration still poses toxicity-related challenges. To address this, we developed the Neovascularized Implantable Cell Homing and Encapsulation (NICHE), a subcutaneous device that enables both allogenic islet transplantation and local, sustained delivery of immunosuppressive drugs (Paez-Mayorga et al., 2022). Although the NICHE has demonstrated promise in supporting islet engraftment, optimal immunosuppressive strategies for long-term graft survival remain to be defined. Costimulatory pathway blockade has emerged as a compelling approach in organ transplantation. In this study, we evaluated the efficacy of locally delivered anti-CD154, alone or in combination with CTLA4-Ig or anti-CD28, in preventing islet rejection and elucidated the resulting immunological profile.

Hypothesis

Local delivery of costimulatory pathway blockers anti-CD154, alone or in combination with CTLA4-Ig or anti-CD28, will prevent islet graft rejection and modulate the local immune microenvironment to support long-term islet survival.

Methods

Immune responses to transplanted islets were evaluated in streptozotocin (STZ)-induced rat model, using both male and female subjects at day 28 post-transplantation. Rats received one of four local treatments via the NICHE platform: (1) anti-CD154 + CTLA-4 Ig, (2) anti-CD154 + anti-CD28, (3) anti-CD154 alone, or (4) no treatment. All groups were given a short systemic course of rapamycin, except for a transplant control group that received no local or systemic immunosuppression. The local immune microenvironment surrounding the transplanted islets in the NICHE was analyzed using mass cytometry (CyTOF). Systemic immune responses were

further assessed in the draining lymph nodes (dLN), spleen, and peripheral blood using flow cytometry. Additionally, fasting C-peptide was measured to assess the effectiveness of each treatment group in enhancing allogeneic islet survival in the NICHE microenvironment.

Results

The combination of anti-CD154 + CTLA-4 Ig resulted in a significant reduction of $CD4^+$ T cells, $CD4^+$ memory T cells, and regulatory T cells (Tregs) in both male and female rats, compared to the untreated and anti-CD154 alone groups. In contrast, the combination of anti-CD154 + anti-CD28 led to a significant increase in Tregs in both sexes relative to control groups. Notably, this regimen also induced a significant reduction in $CD4^+$ effector memory T cells in females, but not in males, indicating a sex-specific immune modulation effect. Flow cytometry analysis revealed no statistically significant differences among the studied groups in systemic immune responses, assessed in draining lymph nodes (dLNs), spleen, and peripheral blood, suggesting that local immunosuppressant delivery does not affect systemic compartments.

Conclusion

These findings demonstrate that the NICHE platform enables localized combinatory immunosuppression to enhance islet graft survival with minimal systemic effects. Notably, sex-specific immune responses were observed, underscoring the need to consider biological sex in optimizing personalized immunotherapy strategies for improved transplant outcomes.

Acknowledgments

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Competing Interests

CYXC, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare they have no competing interests.

#40

hTERT mRNA protects human skin from radiation-induced DNA damage

Thi Kim Cuc Nguyen[1]; David F. Chang[1], Karem A. Court[2], Biana Godin[2], Anahita Mojiri[1], John P. Cooke[1].

[1]Departments of Cardiovascular Sciences and [2]Nanomedicine, Houston Methodist Hospital, Houston, TX 77030, USA.

Introduction: Gene therapy, particularly mRNA therapy, offers significant advantages for disease treatment and vaccine development. However, delivery vehicles must be optimized to ensure both safety and efficacy. Telomerase (hTERT) is a ribonucleoprotein with multiple functions, including extending or repairing telomeres and regulating the transcription of certain genes, thereby enhancing genomic stability and facilitating repair of genetic damage. Although hTERT mRNA can be delivered to cells using various methods, efficient delivery to skin tissue remains challenging with currently available reagents. In this study, we found that MC3 lipid nanoparticles (MC3-LNPs) were effective for delivering hTERT mRNA to human skin.

Method: Human telomerase (hTERT) mRNA was encapsulated in either DOTAP or MC3 nanoparticles and delivered into ex vivo human skin using microneedling 24 hours prior to radiation. The skin samples were then analyzed for hTERT expression, enzymatic activity, and histological changes.

Result: Our data suggest that hTERT was successfully expressed in human ex vivo skin and exhibited enzymatic activity. Notably, hTERT expression markedly reduced phosphorylation of γH2A.X, a key marker of DNA damage, as early as 2 hours after 5 Gy irradiation. Alongside the decrease in γH2A.X, other senescence-associated markers, including p21 and p16, were also reduced. The reduction of γH2A.X following irradiation subsequently led to decreased apoptosis at 24 hours post-radiation.

Conclusion: These findings indicate that hTERT mRNA plays a crucial role in protecting skin from radiation-induced damage, although the precise underlying mechanisms remain to be fully elucidated.

Keyworks: MC3 nanoparticles, Telomerase, Radiation

#41

Assessment of Targeted Immunomodulation in a 3D-Bioengineered Subcutaneous Platform to Improve Islet Graft Survival in Rats

Mia Girela[1,2], Nikita Campa-Carranza, PhD[1,3], Rim Ouni, PhD[1], Corrine Ying Xuan Chua, PhD[1], Alessandro Grattoni, PhD[1,4,5]

[1]Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2]Wiess School of Natural Sciences, Rice University, TX, USA; [3] Tecnologico de Monterrey School of Medicine, NL, Mexico; [4]Department of Surgery, Houston Methodist Hospital, TX, USA [5] Department of Radiation Oncology, Houston Methodist Hospital.

Key words: Islet transplantation, immunomodulation, implantable device, localized immunosuppression

Introduction

Islet transplantation holds potential for restoring glucose control in type 1 diabetes, yet its effectiveness is hindered by the need for toxic, systemic immunosuppressive treatment. To address these challenges, this study utilizes the Neovascularized Implantable Cell Homing and Encapsulation (NICHE) device, a subcutaneous, vascularized platform that enables co-delivery of islets and immunosuppressants directly at the graft site, creating a defined microenvironment for targeted immune modulation (Paez-Mayorga et al., 2022). In this study, we investigated the immunomodulatory potential of anti-CD28, an agonistic antibody known to expand regulatory T cells (Tregs), in combination with short-term systemic rapamycin. We also compared the in vitro effects of combinatorial regimens incorporating anti-CD154 with either anti-CD28 or CTLA4-Ig.

Hypothesis

Local delivery of immunosuppressant combinations through the NICHE platform induces agent-specific effects at the graft-site and systemic compartments.

Methods

In vitro immune modulation was assessed via mixed lymphocyte reaction (MLR) with splenocytes isolated from Lewis (donor) and Fischer (recipient) rats cultured with anti-CD154 + anti-CD28 agonist or anti-CD154 + CTLA4-Ig combinations at 10 or 50 µg/mL each. Proliferation of T cells, Natural Killer (NK) cells, and Tregs was quantified by flow cytometry. For proof-of-concept in vivo studies, healthy Fischer rats were transplanted with Lewis islets into prevascularized NICHE devices. Animals were randomized to receive either localized anti-CD28 delivery through NICHE combined with short course systemic rapamycin or short course systemic rapamycin alone. On day 28 post-transplant, immune profiling was performed on NICHE explants (graft site), spleen, draining lymph nodes, and peripheral blood.

Results

MLR indicated that the combination of anti-CD154 and CTLA4-Ig significantly suppressed immune cell proliferation, including a complete inhibition of regulatory Treg expansion. In contrast, the combination of anti-CD154 and anti-CD28 agonist promoted proliferation of NK and T cells, alongside expansion of the Treg population. In vivo, localized anti-CD28 treatment increased frequencies of Tregs and CD8+ T cells while reducing CD4+ T cells, NK cells, and dendritic cells compared to rapamycin-only controls. Systemic analyses showed minimal immune population changes in the draining lymph node and spleen, though peripheral blood demonstrated decreased CD4+ T cell levels and increased circulating Tregs in the anti-CD28 group.

Conclusion

Localized delivery of anti-CD28 via the NICHE device, in combination with short-term systemic rapamycin, effectively modulated the transplant immune microenvironment by enriching Tregs and altering effector populations. These findings highlight the potential of site-specific drug delivery platforms to achieve targeted immunomodulation and support long-term graft protection in islet transplantation.

Acknowledgements

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Competing Interests

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#42

Biodistribution of Locally Delivered Immunosuppressant Combinations from a Vascularized Subcutaneous Platform for Islet Transplantation

Sumita Dantu[1,2], Rim Ouni, PhD[2], Jocelyn Nikita Campa-Caranza, PhD[2,3], Gabrielle E. Rome[2], Henry Seaborne[2], Elisa Balboni[2,6], Mia Girela[1,2], Corrine Yin Xuan Chua, PhD[2], Alessandro Grattoni, PhD[2,4,5]

[1]Wiess School of Natural Sciences, Rice University, TX, USA; [2]Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [3]School of Medicine and Health Sciences, Tecnologico de Monterrey, NL, Mexico; [4]Department of Surgery, Houston Methodist Hospital, TX, USA; [5]Department of Radiation Oncology, Houston Methodist Hospital; [6]Department of Applied Science and Technology, Politecnico Di Torino, Turin, Italy

Keywords
Islet allotransplantation; Immunosuppression; Biodistribution; Drug delivery

Introduction
Islet allotransplantation represents a promising therapeutic approach for Type 1 Diabetes; however, it is limited by the toxicity of long-term systemic immunosuppression. To address this challenge, localized drug delivery platforms are being explored as means to protect grafts while minimizing systemic exposure. One such approach is the Neovascularized Implantable Cell Homing and Encapsulation (NICHE) device, a vascularized subcutaneous platform that enables simultaneous islet engraftment and sustained local release of immunosuppressive (IS) agents (Paez-Mayorga et al., 2022), offering a unique opportunity to evaluate tissue-level drug biodistribution

Hypothesis
Local co-stimulatory blockade delivered through the NICHE device would result in confined local drug distribution with minimal systemic exposure.

Methods
Streptozotocin (STZ)-induced diabetic rats were transplanted with allogeneic islets into prevascularized NICHE devices. Animals were assigned to one of three local treatment groups: (1) anti-CD154 monotherapy, (2) anti-CD154 + CTLA4-Ig, or (3) anti-CD154 + anti-CD28 agonist. All groups also received systemic rapamycin for 14 days post-transplantation (POD). On POD 28, rats were euthanized for biodistribution analysis. Concentrations of locally delivered agents were quantified in the NICHE tissue, surrounding fibrotic capsule and skin, draining lymph nodes, liver, spleen, kidney, plasma, and the residual drug reservoir.

Results
Across all groups, IS agents demonstrated sustained local presence within the NICHE tissue and fibrotic capsule, with markedly reduced penetration into systemic circulation or off-target organs. This drug retention highlights the NICHE platform’s potential to deliver targeted immunosuppression while reducing systemic exposure and associated potential side effects.

Conclusion

This study provides a detailed characterization of the biodistribution of locally delivered immunosuppressive agents using the NICHE device in a diabetic rat model. By demonstrating that drugs remain concentrated within the transplant microenvironment with minimal systemic spillover, these findings validate the NICHE as a targeted delivery platform capable of reducing systemic drug burden. This work highlights the translational potential of localized immunosuppression strategies to improve the safety profile of islet transplantation and other cell therapies.

Acknowledgements

This study is supported by grant 1R01DK133610-01 from the National Institutes of Health (NIH) and grants SRA-2021-1078-S-B and SRA-2022-1224-S-B from Breakthrough T1D.

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Competing Interests

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#43

Enhancement of NICHE Vascularization and Cellular Engraftment via Peptide-Functionalized PLGA+Gelatin Scaffolds

Gabrielle E. Rome[1], Jocelyn Nikita Campa-Carranza, PhD[1,2], Elisa Balboni, MS[1,3], Purushothaman Kuppan, PhD[4], Henry Seaborne[1], Arya Venugopalan, [1], Rim Ouni, PhD[1], Jesus Paez-Mayorga, MD, PhD[1,2], Corrine Ying Xuan Chua, PhD[1], Gregory Korbutt, PhD[4], Andrew Pepper, PhD[4], and Alessandro Grattoni, PhD[1,5,6]

[1] Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2] Tecnologico de Monterrey, Monterrey School of Medicine, NL, Mexico; [3] Department of Applied Science and Technology, Politecnico Di Torino, Turin, Italy; [4] Department of Immunology and Cell Therapies, Alberta Diabetes Institute, Edmonton, Alberta, Canada; [5] Department of Surgery, Houston Methodist Hospital, TX, USA; [6] Department of Radiation Oncology, Houston Methodist Hospital

Keywords: Cell Transplantation, PLGA, Laminin, VEGF, Bioengineering

Introduction

The NICHE (Neovascularized Implantable Cell Homing Encapsulation) device is designed to establish a highly vascularized and locally immunosuppressed microenvironment conducive to successful cell transplantation. Currently, the NICHE platform employs Mesenchymal Stem Cells (MSCs) to promote vascularization (Paez-Mayorga et al., 2020). Despite their therapeutic potential, MSCs face significant clinical challenges including batch-to-batch variability, limited reproducibility, potential immunogenicity, and limited shelf-life after preparation (Zhou et al., 2021). To address these limitations, we propose the use of a shelf-stable, composite scaffold system composed of polylactic-co-glycolic acid (PLGA) and gelatin, which can be functionalized with Laminin and VEGF to enhance vascularization (Kuppan et al, 2022).

Hypothesis

The PLGA/gelatin scaffold, both in its functionalized and non-functionalized forms, will exhibit comparable vascularization to MSC-loaded NICHE devices in terms of fractional blood volume, capillary area fraction, and capillary density within the cell reservoir.

Methods

The PLGA/gelatin scaffold was wrapped around the NICHE placeholder component before being inserted into the cell reservoir. Devices with non-functionalized scaffolds were stored in phosphate-buffered saline until implantation, whereas devices with functionalized scaffolds were treated with a crosslinking solution to covalently bind bioactive peptides prior to implantation. Devices containing either scaffolds or MSCs were implanted subcutaneously into healthy male rats. At weeks 2, 4, and 6 post-implantation, rats underwent computed tomography (CT) imaging following intravenous administration of liposomal-iodine contrast for vessel visualization. Post-imaging, devices were harvested for histological evaluation of vascularization within the cell reservoir.

Results

Functionalized and non-functionalized scaffold-containing NICHEs exhibited comparable capillary area fraction and capillary density compared to MSC-containing controls across timepoints. At the week 4 timepoint, MSC-loaded devices showed an average capillary area fraction of 0.28% ± 0.074, while functionalized and non-functionalized scaffold-containing devices demonstrated averages of 0.72% ± 0.332 ($p = 0.0674$) and 0.63% ± 0.370 ($p = 0.1735$), respectively, demonstrating a clear trend in favor of the functionalized scaffold. CT imaging corroborated these findings, which revealed no significant differences in fractional blood volume between scaffold-implanted and MSC-loaded devices.

Conclusion

These results suggest that scaffold-based vascularization strategies can effectively replace MSCs, there by mitigating issues related to cellular therapies. An optimal pre-vascularization window of 4 weeks was identified, corresponding to peak performance of the scaffold-containing devices. This timing is critical for subsequent islet transplantation to maximize engraftment and function. Future studies will focus on utilizing scaffold-functionalized NICHE devices in efficacy studies for islet transplantation and localized drug delivery to enhance cell engraftment and modulate immune responses, working towards the development of an optimal microenvironment for cell therapy.

Acknowledgements

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Competing Interests

JPM, CTXC, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare no competing interests.

#44

Short-term Anti-Inflammatory Effects of MCC950 in Tenofovir Alafenamide and Bictegravir Loaded Subcutaneous Implants

Camden Caffey, BS[1], Nicola Di Trani, PhD[1], Ilaria Facchi, MS[1,2], Thi Thao Linh Nguyen, PhD[1], Yongbin Liu, PhD[1], Junhua Mai, PhD[1], Jason Kimata, PhD[3], Junjun Zheng, PhD[4], Corrine Ying Xuan Chua, PhD[1], Alessandro Grattoni, PhD[1,5,6].

[1]Nanomedicine Department, Houston Methodist Research Institute, Houston, TX 77030, USA.
[2]Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy.
[3]Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA.
[4]Immunomonitoring Core, Center for Immunotherapy Research, Houston Methodist Research Institute, Houston, Texas.
[5]Department of Surgery, Houston Methodist Research Institute, Houston, TX 77030, USA.
[6]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA.

Keywords: long-acting drug delivery; subcutaneous implant; foreign body response; NLRP3 inflammasome inhibition; antiretroviral therapy (ART)

Introduction

Subcutaneous implants offer a promising platform for long-acting antiretroviral (LA-ART) therapy, enabling controlled drug release for HIV treatment and prevention. Tenofovir alafenamide (TAF) is highly potent with reduced systemic toxicity, but TAF implants often trigger local inflammation, sometimes necessitating early removal. The combination of TAF with bictegravir (BIC), an integrase inhibitor, is clinically validated in oral formulations and provides robust viral suppression. This study evaluates the short-term anti-inflammatory effects of MCC950, a selective NLRP3 inflammasome inhibitor, when co-delivered via a nanofluidic implant releasing TAF and BIC.

Hypothesis

We hypothesize that co-delivery of MCC950 with TAF and BIC via a nanofluidic implant will reduce local tissue inflammation and improve implant tolerability, without compromising the antiviral activity of TAF and BIC.

Methods

Implants loaded with TAF, urocanic acid (UA, to stabilize TAF), BIC, and MCC950 were incubated at 37°C with agitation for 90 days, and drug release was quantified by HPLC. Subcutaneous devices containing TAF+BIC+MCC950, TAF+BIC, or PBS were implanted in male Sprague Dawley rats. On day 15, fibrotic capsules were excised for histology (H&E and Masson's Trichrome) and immune cell infiltration assessment by imaging mass cytometry.

Results

Co-treatment with MCC950 at 0.1-10 nM did not impair antiviral activity, demonstrating that inflammasome inhibition is compatible with ART. In vitro monitoring demonstrated that the nanofluidic implant provided sustained release of TAF (13.42 ± 6.00 mg), BIC (0.39 ± 0.15 mg), and MCC950 (0.17 ± 0.07 mg), with minimal burst release. TAF+BIC implants in rats induced significant orthogonal swelling (Δ height 0.8 mm), whereas co-delivery of MCC950 reduced Δ height to −0.2 mm, similar to PBS (−0.3 mm), suggesting that MCC950 effectively modulates the foreign body response, reducing tissue swelling and inflammatory response surrounding the implants. At 15 days, MCC950 reduced fibrotic capsule thickness from 129.7 ± 45.98 μm (TAF+BIC) to 99.39 ± 48.65 μm, with histological scores lowered to 8.00 ± 1.41 and preserved angiogenesis. Imaging mass cytometry demonstrated that MCC950 reduced early immune cell infiltration, with decreased total $CD45^+$ leukocytes ($P<0.05$) and $CD8^+$ T cells relative to TAF+BIC ($P<0.01$) alone on day 15.

Conclusions

Co-delivery of MCC950 with TAF and BIC via nanofluidic implants provides sustained antiretroviral release without compromising antiviral efficacy. MCC950 effectively reduces early inflammation, fibrotic capsule thickness, and immune cell infiltration, preserving implant tolerability. These findings demonstrate that selective inflammasome inhibition is a viable strategy to improve the biocompatibility and long-term performance of subcutaneous LA-ART implants.

Acknowledgements

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Conflicts of Interest

Alessandro Grattoni is an inventor of intellectual property licensed by Continuity Biosciences. The other authors declare no conflict of interest.

#45

Sustained Release of Polymer-Antiretroviral Conjugates with Enhanced Solubility and Stability from Nanofluidic Drug Delivery Implant for HIV Pre-Exposure Prophylaxis

Thi Thao Linh Nguyen, Nicola Di Trani, Camden Caffey, Alessio Simeone, Yongbin Liu, Junhua Mai, Corrine Ying Xuan Chua, Alessandro Grattoni
Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA

Keywords: NanoDDI, Sustained release, HIV PrEP, Polymer-Antiretroviral Conjugates

Introduction: With over 1.3 million new infections reported annually, human immunodeficiency virus (HIV) remains a global public health challenge [1]. The development of innovative drug delivery systems is crucial for improving the efficacy and patient compliance of HIV pre-exposure prophylaxis (PrEP) therapies [2]. This study investigates a refillable subcutaneous nanofluidic drug delivery implant (NanoDDI) designed for the sustained release of hydrophobic antiretrovirals, dolutegravir (DTG) and bictegravir (BIC). The NanoDDI technology utilizes Si/SiC nanochannel membranes to achieve sustained drug release via diffusion.
Hypothesis: DTG and BIC are both hydrophobic compounds with poor aqueous solubility and stability, which limit their release from the NanoDDI. To address these limitations, we synthesized conjugates of the drugs and a hydrophilic polymer (HP) to improve their solubility and stability, enabling the sustained release of these compounds from the NanoDDI.
Methods: DTG and BIC were conjugated to a hydrophilic polymer via ester bond formation, yielding HP-DTG (43% DTG) and HP-BIC (37% BIC). Solubility and stability of the conjugates and free drugs were assessed in phosphate-buffered saline (PBS). In vitro release studies were conducted over three months using NanoDDI in PBS, and preliminary in vivo pharmacokinetic studies were performed in rats. High-performance liquid chromatography (HPLC) was used for in vitro analysis, while liquid chromatography–mass spectrometry (LC-MS) was employed for plasma sample analysis.
Results: The conjugates exhibited significantly improved solubility in PBS (39 mg/mL for HP-DTG and 170 mg/mL for HP-BIC) compared to the free drugs. Stability studies conducted at 37°C and 60°C revealed that while the free drugs degraded in PBS, the conjugates remained stable over three months. In vitro release profiles showed sustained drug release, with 44.2% of HP-DTG and 33.7% of HP-BIC released over three months. In vivo, DTG plasma levels were maintained at 4 ng/mL for up to two months, and BIC levels remained at 67 ng/mL after four months. These findings suggest that the NanoDDI, combined with drug-polymer conjugation, is a promising platform for long-acting delivery of hydrophobic antiretrovirals. While the current drug level in plasma did not reach the target thresholds for effective HIV PrEP (64 ng/mL for DTG and 162 ng/mL for BIC), future optimization of nanochannel size and microchannel number of the NanoDDI is expected to achieve therapeutic levels.
Conclusion: The NanoDDI loaded with polymer-drug conjugates represents a viable and effective strategy for the sustained release of HIV PrEP drugs. Additionally, its refillable design eliminates the need for repeated surgical procedures, offering a patient-friendly alternative to traditional implants. This approach has the potential to reduce healthcare costs, improve adherence, and ultimately enhance the overall effectiveness of HIV prevention efforts.
Acknowledgements: This work was supported by NIH grants R01AI120749, R01GM127558 and funding from the Houston Methodist Research Institute.
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#46

Bioengineered Placeholder-Guided Vascular Niche for Enhanced Therapeutic Cell Transplantation

Elisa Balboni, MS[1,2], Jocelyn Nikita Campa-Carranza, PhD[1,3], Gabrielle E. Rome[1], Henry Seaborne[1], Rim Ouni, PhD[1], Corrine Ying Xuan Chua, PhD[1], and Alessandro Grattoni, PhD[1,4,5]

[1]Department of Nanomedicine, Houston Methodist Research Institute, TX, USA; [2]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy; [3]Tecnologico de Monterrey School of Medicine, NL, Mexico; [4]Department of Surgery, Houston Methodist Hospital, Houston, TX, USA; [5]Department of Radiation Oncology, Houston Methodist Hospital

Keywords: Islet transplantation, 3D-printed placeholder, Localized immunosuppression, Transcutaneous drug delivery, Cell-based therapies

Introduction

Type 1 Diabetes (T1D) results from autoimmune destruction of pancreatic β-cells, leading to lifelong dependence on exogenous insulin. Islet transplantation offers a promising approach to restore endogenous insulin secretion. However, the clinical route via portal vein infusion triggers inflammatory responses, hypoxia and shear stress, reducing long-term success. Subcutaneous transplantation proposes a safer, more accessible alternative but is hindered by poor vascularization and islet loss. The Neovascularized Implantable Cell Homing and Encapsulation (NICHE) device addresses these limitations by providing a prevascularized subcutaneous platform with localized immunosuppressant delivery (Paez-Mayorga et al., 2022). Islets transplanted through simple injection remain confined to a narrow region, limiting vascular integration and viability. Creating a broader pre-formed space may facilitate islet spreading, improving engraftment, vascularization, and survival (Wang L-H et al, 2024).

Hypothesis

A temporary 3D-printed placeholder will guide formation of a vascularized cavity in the NICHE, improving islet distribution, vascularization, and survival.

Methods

Placeholders were 3D printed in nylon or resin and characterized in vitro to evaluate surface properties, focusing on cell adhesion and tissue compatibility. For in vivo studies, NICHEs were implanted subcutaneously with resin placeholder inserted in the cell reservoir (SPH group) or without a placeholder (control group). Diabetes was induced with streptozotocin two weeks later. All NICHEs prevascularized for 5 weeks. In the SPH group, placeholders were explanted to create a defined cavity, into which syngeneic islets were transplanted. In the control group, islets were directly injected into the prevascularized reservoir. Blood glucose was monitored for 45 days to assess graft function. At day 14 and 45, NICHEs were harvested for flow cytometry and histological evaluation. Whole-device light sheet imaging was performed to generate 3D reconstructions of islet dispersion and integration within the NICHE.

Results

In vitro material characterization demonstrated that nylon promoted cell adhesion and tissue ingrowth, making it suitable for the NICHE outer structure, whereas the smooth surface of resin minimized adhesion and enabled straightforward removal, making it suitable for the placeholder. In vivo, NICHEs implanted with placeholders developed a well-defined vascularized cavity during the prevascularization period, while controls without placeholders did not form discernible space. Following islet transplantation, transplanted rats maintained normoglycemia for 30 days. Histology revealed that the SPH group exhibited improved islet dispersion, reduced local inflammation, and enhanced integration with the tissue compared to controls. Whole-device light sheet imaging at day 45 further demonstrated uniform islet distribution and stable engraftment within the vascularized cavity.

Conclusion

Integration of a temporary 3D-printed placeholder into the NICHE generates a vascularized cavity that enhances subcutaneous islet transplantation. This placeholder-guided strategy improves islet dispersion, vascular support, and immune compatibility, leading to graft survival and function while reducing local inflammation. This work positions the NICHE as a promising platform for cell-based therapies in T1D and underscores the potential of prevascularization strategies to overcome current limitations in extrahepatic transplantation. Further research will refine the device architecture, optimize material selection, and evaluate long-term efficacy in allogeneic transplantation models to advance towards clinical translation.

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Competing Interests

CYXC, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare no competing interests

#47

Long-Term Sustained Release of MK-8527 from a Refillable Nanofluidic Implant for HIV Pre-Exposure Prophylaxis

Thi Thao Linh Nguyen, Nicola Di Trani, Camden Caffey, Alessio Simeone, Annarita Congedo, Yongbin Liu, Corrine Ying Xuan Chua, Alessandro Grattoni
Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA

Keywords: MK-8527, NanoDDI, HIV PrEP, Sustained release, Pharmacokinetics

Introduction: Despite significant advances in prevention and treatment, human immunodeficiency virus (HIV) continues to pose a major global public health challenge, with an estimated 40.8 million people living with HIV worldwide as of 2024 [1]. Long-acting antiretroviral delivery systems are a promising strategy to improve adherence and efficacy of HIV pre-exposure prophylaxis (PrEP) [2]. MK-8527 is a novel nucleoside reverse transcriptase translocation inhibitor with the potential for extended-duration dosing, making it a good candidate for long-acting HIV PrEP [3]. This study investigates the use of a refillable subcutaneous nanofluidic drug delivery implant (NanoDDI) for the sustained and tunable release of MK-8527.

Hypothesis: MK-8527, with the aqueous solubility of approximately 400 μg/mL, is suitable to be delivered in a controlled, long-term manner via diffusion through the nanofluidic membrane. The modulation of microchannel availability enables tunable release rates suitable for HIV PrEP.

Methods: In vitro release studies were conducted over 12 months using refillable NanoDDIs with fully opened membrane loaded with MK-8527. De-escalation studies assessed release rates by varying the number of opened microchannels (1/8, 1/4, and 1/2 membrane opened). MK-8527 concentrations were determined by high-performance liquid chromatography (HPLC). For in vivo pharmacokinetic (PK) studies, MK-8527-loaded NanoDDIs were subcutaneously implanted in rats. Plasma concentrations were monitored for 3 months (short-term PK) or up to 2 years (longterm PK), and MK-8527 levels were quantified by liquid chromatography–mass spectrometry (LCMS).

Results: MK-8527 demonstrated sustained in vitro release over 12 months, with a daily release rate of 270 μg. De-escalation studies confirmed that reducing the number of microchannels proportionally decreased the release rate, with release rates of 150, 76, and 39 μg/day for the 1/2, 1/4, and 1/8 membrane groups, respectively. The short-term PK study showed sustained plasma levels of MK-8527, with an average release rate of 400 μg/day. In the long-term study, MK-8527 remained detectable in plasma for up to 1 year, supporting the feasibility of long-acting delivery. The study is ongoing, and MK-8527 is expected to remain detectable in rat plasma for at least 2 years.

Conclusion: These findings support the use of refillable nanofluidic implants for long-term, tunable delivery of MK-8527. This platform holds promise for improving adherence and efficacy in HIV PrEP, with potential for personalized dosing strategies.

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#48

Evaluation of Combinatorial Localized Immosupression in NICHE Platform for Long-term Islet Allotransplantation

Henry Seaborne[1], Nikita Campa-Carranza, PhD[1,2], Gabrielle Rome[1], Rim Ouni, PhD[1], Elisa Balboni, MS[1,3], Jesus Paez-Mayorga, MD, PhD[1], Corrine Ying Xuan Chua, PhD[1], and Alessandro Grattoni, PhD[1,4,5]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Texas, USA; [2]School of Medicine and Health Sciences, Tecnologico de Monterrey, NL, Mexico; [3]Department of Applied Science and Technology, Politecnico di Torino, Torino, Italy; [4]Department of Surgery, Houston Methodist Hospital, Texas, USA; [5]Department of Radiation Oncology, Houston Methodist Hospital.

Key words: Type-1 Diabetes, Immunosuppression, Allogenic, Islet, Euglycemia

Introduction
The Neovascularized Implantable Cell Homing and Encapsulation (NICHE) platform is a 3D-printed polymeric device designed to support cell transplantation through direct vascularization and local immunosuppressant delivery. This approach aims to prevent immune rejection while minimizing systemic toxicity. Preclinical studies have demonstrated the NICHE's ability to support islet engraftment; however, optimization of local drug combinations remains essential for longstanding glycemic control. In this study, we evaluated the localized delivery of anti-CD154, CTLA4Ig, and anti-CD28 agonist, each previously shown immunomodulatory effects in transplantation models (Zhang T et al., 2015, Pinelli DF et al., 2013, Benhamou PY et al. 2002).

Hypothesis
Localized delivery of immunosuppressant cocktails within the NICHE platform improves islet engraftment and reduces immune rejection, maintaining long-term glycemic control in diabetic rats.

Methods
Male and female streptozotocin-induced diabetic rats were subcutaneously implanted with NICHE devices. Following a four-week pre-vascularization period, allogeneic islets were loaded into the NICHE. Euglycemia was maintained with subcutaneous insulin pellets during this time. Induction immunosuppression started one day prior to transplantation with systemic rapamycin (1 mg/kg, subcutaneous) and continued for 2 weeks. Animals were randomized into three local treatment groups: anti-CD154 alone, anti-CD154 + CTLA4-Ig (IS1), and anti-CD154 + anti-CD28 agonist (IS2). Controls included islet-only transplants and diabetic rats without treatment. Therapeutic efficacy was evaluated through daily blood glucose (BGL) monitoring, intraperitoneal glucose tolerance tests (IPGTT), serum C-peptide, and histology. Rats were followed until D135 post-transplant or terminated if hyperglycemia recurred.

Results
Experimental rats had improved glycemia for up to 135 days, while diabetic controls reverted to hyperglycemia by day 24, unveiling the duration of the insulin pellets. No significant differences emerged between immunosuppressant groups. However, sex-specific trends were observed: males gained weight more rapidly than females (slope 1.43 ± 0.54 vs. 0.51 ± 0.19, $p < 0.001$),

potentially explaining more stable glycemia in females. Of rats kept for long term survival (post D28 of transplant), 81% of females maintained euglycemia beyond 115 days vs 11% in males.

Day 56 IPGTT confirmed islet function: diabetic controls remained hyperglycemic (AUC = 82,465 ± 3,409 vs. healthy 26,811 ± 1,725, $p < 0.001$). All IS groups improved glucose kinetics (AUC a-CD154 = 48,770 ± 4,271; IS1 = 54,216 ± 3,967; IS2 = 55,977 ± 4,180, $p < 0.001$ vs. control), though none reached healthy control levels. Serum C-peptide increased significantly from non-quantifiable levels post-transplant (a-CD154 0.36 ± 0.15 ng/mL, IS1 1.10 ± 0.24, IS2 1.30 ± 0.29, $p < 0.05$ vs. baseline) but remained below healthy controls ($\bar{x}$ C-pep CTRL = 3.53 ng/mL), with no differences among IS groups. Immunosuppressants remained confined within the NICHE, limiting systemic exposure.

Conclusions

The NICHE platform supported long-term euglycemia and partially restored insulin production in diabetic rats across all immunosuppressant combination groups, with sex-specific differences in weight gain and glycemic stability. Although none of the regimens fully recapitulated healthy controls, the observed improvements in glycemia, glucose tolerance, and C-peptide levels highlight the translational potential of combinatorial local immunosuppression for islet transplantation.

Acknowledgements

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Disclosure Information

JPM, CYXC, and AG are inventors of intellectual property licensed by Continuity Biosciences. AG is a co-founder and scientific advisor of Continuity Biosciences. All other authors declare no conflict.

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#49

A Multi-Species Computational Model for Islatravir: Simulating Long-Acting Strategies Across Administration Routes for Preclinical and Clinical Translation

Alessio Simeone[1, 2], *Marco Maria Paci*[1, 3], *Nicola Di Trani*[1], *Simone Capuani*[1], *Corrine Ying Xuan Chua*[1, 4, 5], *Alessandro Grattoni*[1, 4, 5*].

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA;

[2]Department of Mechanics and Aerospace Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy;

[3]College of Engineering, Swansea University, Swansea, SA1 8EN, Wales, UK

[4]Department of Surgery, Houston Methodist Research Institute, Houston, TX 77030, USA;

[5]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA;

*Correspondence: agrattoni@houstonmethodist.org (A.G.); Tel.: +1-(713)-441-7324 (A.G.)

Keywords: computational model, pharmacokinetics, Islatravir, simulation, antiretroviral drug.

Abstract

The development of effective and safe HIV prevention strategies remains a critical global priority. Islatravir (ISL), a novel nucleoside reverse transcriptase inhibitor (NRTI), has emerged as a promising long-acting pre-exposure prophylaxis agent (LA-PrEP), due to its extended intracellular half-life of ~209 h[1]. Adverse effects, like lymphocyte count reduction in subjects exposed to high doses, have halted further investigational clinical trials[2]. We developed a computational model to simulate ISL plasma and active ISL-triphosphate in peripheral blood mononuclear cells (PBMCs) and to predict outcomes of various administration routes, such as oral bolus, intravenous injection and zero-order release subcutaneous implant developed in our laboratory (NanoDDS)[3], in three different species, Sprague-Dawley rat, rhesus macaque and human. The purpose of this study is to bridge preclinical-to-clinical translation and to offer a valuable platform for refining ISL delivery systems to maximize HIV risk reduction while minimizing toxicity.

The model was built on MatLab's platform SimBiology and relies on a series of parameterized ordinary differential equations (ODEs), each describing transfer rates of the drug between major body compartments. Parameters of each equation have been found through literature data fitting, when such data was available, or allometrically scaled between species, constrained with reasonable physiological assumptions. Intracellular uptake of ISL was modeled through a Michaelis-Menten equation to better describe saturation effect of ISL intracellular transporters, common outcome if the administration method is sustained.

We were able to correctly match the biphasic curve of ISL plasma concentration for oral (rhesus macaques and humans) and intravenous bolus (rats) administrations, as well as steady-state concentrations when using our subcutaneous implant. We used once-daily and once-monthly oral dosing in humans as independent validation sets.

Even though it is a deterministic model that does not consider yet intraspecies or sex-related variability and given the lack of experimental data, this work represents an important milestone in the attempt to fully computerize design and development of subcutaneous drug delivery systems and a significant step towards animal experimentation reduction. Further experimental data collection and in-vitro and in-vivo studies are planned to enrich and additionally refine the model to be even more accurate or capable of describing drug distribution also in other organs. A coupled dissolution/diffusion model is also scheduled to better understand the drug dynamics inside the implant.

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#50

Title

Formulation development of the antiretroviral MK-8527 for a Long-Acting Refillable Nanofluidic Implant.

Annarita Congedo[1,2], Nicola Di Trani[1], Thi Thao Linh Nguyen[1],Camdem Caffey[1], Alessio Simeone[1], Alessandro Grattoni[1,3,4]

[1]Houston Methodist Research Institute, Houston, TX, United States

[2] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy,

[3]Department of Surgery, Houston Methodist Hospital, Houston, TX, USA,

[4]Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX, USA

Keywords

Drug delivery, Nanomedicine, HIV, Sustained release, Subcutaneous implant

Introduction

Long-acting antiretroviral therapies are essential to reduce viral load to undetectable levels, preventing progression and transmission.

In order to allow this, a subcutaneous implantable device (NanoDDS) enables continuous drug release from a rapid single hand injection. It consists of a drug reservoir, a porous nanochannel membrane, a titanium filter, and two self-sealing silicon rubber ports. Interstitial fluids permeate the nanochannels, dissolving the drug, which is then released by diffusion, with the release rate controlled by the size and number of channels.

Hypothesis

Sustained release is often limited by drug aggregation and compaction inside the device, especially at higher loads. We hypothesize that optimized formulations of MK-8527 incorporating anticaking excipients can prevent compaction during refilling, improve solubility, and enable long-term, consistent drug release.

Methods

Formulations containing MK-8527 with different excipients were prepared and evaluated. Quantitative analyses were first carried out to identify the most promising candidates, followed by qualitative tests assessing drug accumulation inside the device and injection force. A syringe pump was used to standardize filling conditions at different flow rates, ensuring leakage prevention and maintaining port integrity.

Results

Among the anticaking agents tested, sugars improved solubility and reduced injection force. To further evaluate performance, devices were loaded with five different drug quantities. At the highest drug load, formulations including an anticaking agent prevented aggregation and maintained release functionality.

Conclusions

Preliminary findings indicate that optimized MK-8527 formulations enabled 6-month sustained and consistent drug release from the NanoDDS implant. Ongoing in vitro studies and recently initiated in vivo evaluations will further confirm the potential of this approach for long-term HIV treatment.

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#51

Title: MMP-2-Targeting Peptide Amphiphiles as a Vehicle for Treatment of Aortic Aneurysms

Authors: Benjamin T. Ledford Ph.D. [1], Mark R. Karver Ph.D. [2], Liam C. Palmer Ph.D. [2, 5], Dhwanit Dave Ph.D. [2], Kyle Gray B.S. [6], David C. Gillis M.S. [1], Hai Xu M.S. [1], Samuel I. Stupp Ph.D.[2, 5-8], Nick D. Tsihlis Ph.D.[1, 3], and Melina R. Kibbe M.D. [3,4]

1. Department of Surgery, University of Virginia
2. Center for Regenerative Nanomedicine, Northwestern University
3. Department of Biomedical Engineering, University of Virginia
4. Department of Cardiothoracic & Vascular Surgery, University of Texas Health Houston
5. Department of Chemistry, Northwestern University
6. Department of Materials Science and Engineering, Northwestern University
7. Department of Biomedical Engineering, Northwestern University
8. Department of Medicine, Northwestern University

Introduction: Abdominal aortic aneurysm (AAA) is a localized dilation of the aorta in the abdomen that poses a severe risk of mortality when ruptured. The cause of AAA is not fully understood, but degradation of medial elastin due to elastolytic matrix metalloproteinases (MMPs) is a key step in disease progression. Current therapeutic interventions are limited to surgical repair. We have previously demonstrated that intravenous injectable peptide amphiphile (PA) supramolecular nanofibers target MMP-2 in a rodent model of AAA.[1] This study aims to investigate MMP-2-targeting PA nanofibers as a delivery vehicle for therapeutic compounds to treat AAA.

Hypothesis: Intravenous administration of MMP-2-targeting PA nanofibers will deliver therapeutic compounds to the aneurysmal niche and prevent AAA development.

Methods: Solid-phase peptide chemistry and high-performance liquid chromatography (HPLC) were used to synthesize and purify peptide-drug conjugates containing 3-phenyl-2-[(4-phenylphenyl)sulfonylamino]propanoic acid (3PSPA), which is a potent MMP2/9 inhibitor. *In silico* molecular dynamics modeling was used to determine the range at which the peptide-3PSPA can be incorporated into nanofiber constructs. Transmission electron microscopy (TEM) and small-angle X-ray scattering (SAXS) measurements were used to characterize nanostructure morphology, and circular dichroism (CD) spectroscopy and wide-angle X-ray scattering (WAXS) analysis revealed secondary peptide structure. Nanofiber gels were used to assess therapeutic release by quantifying the concentration of 3PSPA via its spectral properties. *In vitro* efficacy of peptide-3PSPA was investigated using gelatin zymography. To evaluate *in vivo* efficacy, therapeutic MMP-2-targeting PA nanofibers were administered 2x/week at 12mg/kg in a rat model of AAA and aortic dilation over 14 days was measured using ultrasonography. Weight loss was used as a proxy for biocompatibility.

Results: Peptide-drug conjugates were >95% by HPLC. Coarse-grained molecular dynamics simulations equilibrated in three independent 10 μs trajectories showed that peptide-3PSPA conjugates incorporated into nanoscale PA structures. Interestingly, TEM and SAXS showed that nanofiber diameter increased as ratios of peptide-drug to PA increased. CD spectroscopy and WAXS of nanofiber co-assemblies containing MMP-2-targeting PA and peptide-3PSPA showed expected characteristics of β-sheet formation. Peptide-3PSPA was gradually released from therapeutic MMP-2-targeting PA nanofibers over time at a range of co-assembly ratios. Gelatin zymography assays demonstrated strong MMP-2 inhibitory activity with the peptide-3PSPA conjugate (IC_{50} ~1.7 μM n=3) that was comparable to unmodified 3PSPA (0.8 μM).[2] Finally, ultrasound data revealed a significant decrease in aortic dilation in rats treated with MMP-2-targeting PA nanofibers carrying peptide-3PSPA versus controls (13.8±3.3% vs. 31.8±6.1%, n=3, $p<0.001$) without any associated weight loss.

Conclusions: Molecular simulations, TEM, CD spectroscopy, and X-ray scattering data demonstrate successful incorporation of peptide-drug conjugates into MMP-2-targeted PA nanofibers. Critically, *in vitro* data and early animal studies support the use of the peptide conjugated MMP-2 inhibitors as a viable strategy for limiting aortic dilation in a rodent model of AAA.

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#52

Optimizing saRNA for Delivery of a Therapeutic Protein

Cynthia Huang[1,2] , Deanna M. Santer[3], Warren W. Wakarchuk[4] and Anna Blakney[1,2]

1 The University of British Columbia, School of Biomedical Engineering
2 The University of British Columbia, Michael Smith Laboratories
3 Department of Immunology, University of Manitoba
4 Department of Biological Sciences, University of Alberta

Keywords: Therapeutic protein, saRNA

Self-amplifying RNA (saRNA) is primarily used for prophylactic purposes and is currently approved for use in COVID vaccines in Japan, Europe, and India.[1] A replicase encoded in saRNA performs *in situ* amplification of the subgenomic region, which allows for higher and sustained expression for.[2] Self-amplifying RNA (saRNA) can be administered at doses in orders of magnitude lower than mRNA for vaccines.[2] A SARS-COV-2 saRNA vaccine approved in Japan (ARCT-154) is administered at a dose of 5 μg, which is 6-20-fold lower than approved mRNA COVID-19 vaccines.[2] Lower doses of saRNA have demonstrated longer protein expression lasting weeks beyond mRNA therapeutics.[2] This makes saRNA a good candidate for the delivery of a therapeutic protein, which requires a higher concentration of the functional protein compared to a vaccine antigen. However, it is necessary to optimize saRNA formulations specifically for the delivery of a therapeutic protein by avoiding immune detection and excess inflammation.

Here, saRNA constructs were designed to encode a viral replicase and a reporter protein attached to a therapeutic protein through a self-cleaving peptide. *In vitro* luciferase assays, qPCR, ELISA, and secondary stimulation in an epithelial cell line were performed to analyze the functionality of the designed constructs.

Initially, using saRNA to express secreted therapeutic proteins resulted in minimal replication and translation when the secreted protein was placed before the P2A and firefly luciferase reporter. Switching the order of the two genes in the construct resolved replication and translation issues. The presence of a reporter gene resulted in a ~50% reduction in replication, compared to a construct with the therapeutic protein alone. Replication of the reporter and the therapeutic protein was not significantly different. An ELISA performed on the cell supernatant of HEK293T cells reflected the same trend, where the constructs with only the therapeutic protein had 2-3X the concentration of therapeutic protein produced compared to a construct with a reporter. Stimulation of epithelial cells with the cell supernatant containing the therapeutic protein resulted in no upregulation of TNF-α, which is a major proinflammatory mediator.[3]

This work is necessary for understanding the different considerations needed to design saRNA for expressing therapeutic proteins effectively and preventing excess immune activation, compared to a vaccine, where an immune reaction is desired. Levels of therapeutic protein vary when attached to a reporter, which may matter less in a vaccine context. This necessary to note when performing studies that contain a reporter during experimental planning. Moreover, using saRNA for replication of various secreted proteins may require further adjustments to ensure functional replication or translation. This is not typically an issue for co-expression of two intracellular proteins. Further studies in vivo will be performed to analyze biodistribution and function of the therapeutic protein.

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#53

Physicochemical Determinants of Drug Retention and Release in Poly(2-oxazoline) Micelles.

*Alyssa Holden[1, 2], *Liyuan Luo[1, 3], Hallie Hutsell[1, 2], Jacob Ramsey[2, 4], Alexander Kabanov[1, 2, 5]

[1] *Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina, USA*

[2] *Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina, USA*

[3] *College of Arts & Sciences, University of North Carolina, USA*

[4] *Department of Surgery, Duke University School of Medicine, USA*

[5] *Lineberger Comprehensive Cancer Center, University of North Carolina, USA*

**Indicates co-first author*

Nanomedicine is transforming cancer therapy by enabling precise delivery of drugs to tumors while reducing systemic toxicity. Among these strategies, poly(2-oxazoline) (POx) polymer micelles are especially promising as they solubilize hydrophobic drugs at higher drug loading than other carriers, improve circulation time, and enhance tumor accumulation through nanoscale design[1]. Co-encapsulating multiple drugs at synergistic ratios within a single micelle and controlling their release profiles holds great promise for enhancing cancer treatment efficacy. However, there remains a limited understanding of how to rationally optimize complex micelle formulations to achieve desired retention and release characteristics, creating a critical gap in the design of effective nanomedicines. In this study, we developed a library of POx micelle formulations co-loading paclitaxel and metronidazole benzoate with immunologically active drugs to target oncogenic bacteria resident within tumors. We hypothesized that differences in physicochemical properties would dictate drug retention and release through drug–drug and drug–polymer interactions, and that these effects could be captured by a physiologically informed model linking *in vitro* measurements to *in vivo* pharmacokinetics. To this end, we measured the critical micelle concentration (CMC) to assess stability, the partial specific volume to account for true micelle-associated drug volume, and partitioning coefficients to quantify thermodynamic drug distribution, together enabling predictive connections between micelle formulation and pharmacokinetic behavior. Ultimately, we aim for this study to provide insights into how structural and physicochemical features of drugs govern micelle retention and release, knowledge that can be leveraged to rationally design future formulations and accelerate the development of new drug candidates. This approach has the potential to streamline the product pipeline and improve the clinical success of future therapeutic innovations.

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#54

Synthesis and characterization of alendronate-coated PLGA nanoparticles loaded with all-trans retinoic acid for prostate cancer bone metastasis

Keywords: all-trans retinoic acid, alendronate, PLGA nanoparticle, prostate cancer bone metastasis, endothelial-to-osteoblast transition

Kitz Paul Marco[1,2], Allan John Barcena, MD, PhD[1], Marvin Bernardino[1], Anna Marks[1,3], Guoyu Yu, PhD[4], Francisco Heralde III, PhD[2], Sue-Hwa Lin, PhD[3,4,5], Marites Melancon, PhD[1,3]

[1] Department of Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[2] College of Medicine, University of the Philippines Manila, Manila 1000, Philippines
[3] The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, TX 77030, USA
[4] Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[5] Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

Introduction: All-trans retinoic acid (ATRA) is a promising drug for prostate cancer bone metastasis with multifaceted effects in the bone microenvironment. It directly inhibits metastatic prostate cancer cells and suppresses tumor-induced endothelial-to-osteoblast transition, a key driver of aberrant bone formation, tumor growth, and pro-tumorigenic immune modulation. However, ATRA's clinical utility is limited by poor pharmacokinetics and toxicity at high doses. Nanoparticles (NPs) can enhance drug delivery by improving circulation, bypassing biological barriers, and enabling selective accumulation at target sites though surface functionalization. Poly-(lactic-co-glycolic acid) (PLGA) NPs are particularly attractive due to its biocompatibility, tunability, and favorable degradation kinetics.

Hypothesis: We hypothesize that encapsulating ATRA in a PLGA NP that is coated with alendronate, a bone-targeting drug, will improve delivery to osteoblastic prostate cancer and subsequently its efficacy.

Methods: PLGA NPs co-loaded with ATRA and indocyanine green (ICG) were synthesized using an oil-in-water emulsion method and coated with a crosslinker-conjugated alendronate. This targeted NP is termed as ATRA-ICG-PLGA-ALN NPs. The near-infrared dye ICG was added to enable tracking. Non-targeted NPs without alendronate (ATRA-ICG-PLGA NPs) were also synthesized as controls. The NPs were characterized for size, polydispersity, zeta potential, drug loading, and release profile. In vitro binding to hydroxyapatite and prostate cancer-induced aberrant bone formation were assessed using spectroscopy and fluorescence microscopy, respectively. Finally, the effects of nanoparticle encapsulation on the pharmacological activities of ATRA on prostate cancer cytotoxicity and endothelial-to-osteoblast transition were determined using the alamarBlue assay and Alizarin red staining, respectively.

Results: ATRA-ICG-PLGA-ALN NPs were larger (330.0 ± 50.92 nm vs 263.0 ± 1.276 nm) and more negatively charged (-7.390 ± 5.519 mV vs -4.147 ± 3.134 mV) than non-targeted NPs. Meanwhile, drug loading was similar (2.93% ± 0.04% vs 2.63% ± 0.01%). Both NPs showed controlled drug release, with only <20% of the drug released within 48 hours, while ATRA release was slower in ATRA-ICG-PLGA-ALN NPs (19.02% ± 2.065% vs 11.27% ± 1.435% after 48 hours). Alendronate functionalization significantly increased hydroxyapatite binding from 6.37% ± 3.09% to 65.7% ± 1.91% ($p<0.0001$) and promoted selective binding to calcified matrix derived from BMP4-treated 2H11 endothelial cells. NP encapsulation also appeared to improve

ATRA's bioactivity in vitro. ATRA-ICG-PLGA-ALN NPs reduced PC3 cell viability significantly more than free ATRA (37.08% ± 7.763% vs 55.07% ± 11.04%, $p<0.05$) while non-targeted NPs exhibited comparable cytotoxicity to the free drug (61.45% ± 18.99%). Both NPs resulted in visually reduced BMP4-induced bone formation in 2H11 cells compared to free ATRA.

Conclusions: In summary, ATRA-ICG-PLGA-ALN NPs can selectively bind bone matrix, slowly release the drug, and enhance the inhibitory effects of ATRA on prostate cancer viability and pathologic tumor-induced bone formation in vitro. Hence, this bone-targeted nanoparticle could enhance the delivery of ATRA to osteoblastic prostate cancer bone metastasis and improve its safety and efficacy. Future in vivo studies will be conducted to further validate the therapeutic potential of this platform.

#55

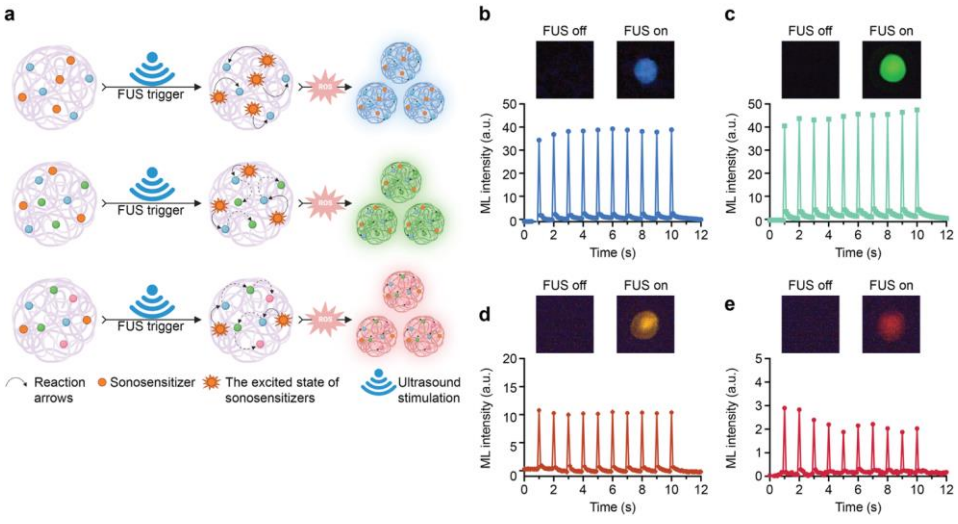
Wavelength-Programmable Mechanoluminescent Nanoparticles for Ultrasound-Gated, Multispectral Optogenetic Control

Xiangping Liu

Biomedical Engineering Cockrell School of Engineering, The University of Texas at Austin, Austin, Texas 78712, United States.

Keywords: Ultrasound, sonosensitizers, mechanoluminescence, sono-optogenetics

Precise, non-invasive optical control of neural circuits requires mechanoluminescent emitters whose spectra align with the action windows of opsins beyond channelrhodopsin-2 (ChR2). Here, we introduce a wavelength-programmable platform of organic mechanoluminescent nanoparticles (OMLNPs) that spans the visible spectrum through sequential Förster resonance energy transfer (FRET). Green-emitting fluorescein-β-cyclodextrin nanoparticles (FL-βCD NPs) served as the core donors. A two-step FRET cascade—energy release from the chemiluminophore L012 to fluorescein, followed by transfer to secondary acceptors Eosin Y (EY), Phloxine B (PB), or Rhodamine B (RhB)—red-shifted the emission to longer wavelengths. The resulting series (L012-βCD, FL-βCD, EY-βCD, PB-βCD, and RhB NPs) displayed chemiluminescence maxima at 459, 524, 550, 573, and 592 nm, respectively. Under pulsed ultrasound, each formulation produced temporally synchronized mechanoluminescent flashes—blue, green, yellow, orange, and red—mirroring its spectral design. Quantitative analysis showed a monotonic decrease in emission intensity with red-shifting, with FL-βCD NPs yielding the brightest output. Leveraging this tunability, we successfully coupled specific OMLNP wavelengths to opsin activation: ChR2 and ChRmine for excitation and eOPN3 for inhibition. Collectively, these OMLNPs provide a versatile, ultrasound-gated toolkit for multiplexed excitation or inhibition of diverse opsins, paving the way for deep-tissue, multichannel optogenetic modulation.



ML characterization of multicolor L012-based nanoparticles.

#56

Single Molecule DNA Tweezers Enable Programmable Control of Enzyme Activity Via Arbitrary Molecular Cues

Sara Nixon, Shivudu Godhulayyagari and Devleena Samanta

University of Texas at Austin

Keywords: Enzyme, DNA, Allosteric, Tweezer

Developing enzyme therapeutics which are conditionally activated by specific molecular triggers would allow for targeted effects. The mechanism of allostery, wherein an effector binds and modulates enzyme activity, is ideal for establishing these therapeutic pathways. However, engineering allosteric control sites into enzymes is non-trivial; it typically requires extensive protein modification, which can effect structure and function. To overcome this barrier, we have introduced single-molecule DNA tweezers (SMDTs), a platform that enables programmable, clinically relevant regulation of enzyme activity in response to user-defined molecular cues, without altering the enzyme itself. SMDTs consist of two aptamers connected with a stimuli-responsive DNA linker. The SMDT adopts a “pinched” conformation that suppresses enzymatic activity via bivalent binding to two epitopes of the enzyme. Upon exposure to therapeutic triggers, the SMDT should undergo a conformational change that releases thrombin, restoring function and enabling control of clotting activity, which can be readily monitored via fibrinogen functional turbidimetric assay (FIFTA). The degree of inhibition and reactivation can be finely tuned by adjusting the DNA linker’s length, sequence, and flexibility, offering programmable control. SMDTs with a range of linker lengths (10-60 nts) were tested, with SMDTs containing a 15-nt ssDNA linker showing the greatest reduction in thrombin activity. SMDTs with linkers ranging from 10, 20, 30, 40, 50, and 60-nts also produced strong inhibition at higher concentrations. Thrombin activity was restored via nucleic acid inputs (RNA and DNA), in a hybridization, sequence-specific manner. For non-nucleic acid inputs, the linker region was redesigned to incorporate an aptamer or other recognition motif with specific binding to the target cue. SMDTs responsive to cancer-related biomarkers such as TATA-binding protein (TBP), platelet-derived growth factor (PDGF), and c-Myc were successfully engineered. Additional SMDTs were designed to regulate thrombin activity in response to kanamycin and Mn^{2+} ions. In each case, thrombin activity was restored upon exposure to the programmed input. By demonstrating regulation with diverse therapeutic triggers, including nucleic acids, transcription factors, signaling proteins, and small molecules, we have established a framework for translating molecular recognition into therapeutic enzyme control with potential for targeted delivery and therapeutic action.

Acknowledgments: This work is supported by start-up funds from The University of Texas at Austin, National Science Foundation (NSF CHE 2404334), the Packard Foundation (Grant #2024-77398), and the Welch Foundation (F-2209-20240404).

#57

Deciphering Extracellular Vesicle Uptake Mechanisms in Ovarian Cancer: Insights into Tropism and Resistance Transfer

Joana Sousa Esteves [1,2], Kadie Edwards [1], Jetzabel Garcia-Parra [1], Claire Donnelly [1], Francesca Taraballi [2], Deyarina Gonzalez [1]

[1] Reproductive Biology and Gynaecological Oncology Group, Swansea University Medical School, Singleton Park, Swansea SA2 8PP, UK

[2] Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, 77030, Texas, USA

Introduction: Ovarian cancer is a leading cause of death in women worldwide. In the US, 20,000 new cases and 12,000 deaths are projected for 2025. A variety of therapeutic approaches are available including widely used platinum-based chemotherapeutic drugs, nonetheless the average 5-year survival rate is 51.6% and it remains the deadliest gynecological cancer in the US. Furthermore, there are increasing rates of chemotherapy resistance which are directly linked to poorer outcomes. There is an urgent demand for more effective strategies to increase survival rates. Extracellular vesicles, nanosized particles released by cells, play a role in intercellular communication. In ovarian cancer they have been shown to play a role in the progression of disease, contributing to an aggressive phenotype and increasing cancer cell proliferation, seemingly contributing to the development of chemotherapy resistance.

Methods: This study investigates EV uptake mechanisms in ovarian cancer (OC) cell lines, comparing tumor-derived EVs from chemotherapy-sensitive (SensC-EVs) and resistant (ChemoRC-EVs) cell lines. EVs were isolated via size exclusion chromatography from serous subtype cell lines, SKOV3 wild type, SKOV3 cisplatin-resistant (SKOV3 cis); and endometroid subtype cell lines, A2780 wild type, and A2780 cisplatin-resistant (A2780 cis). EVs were characterized by nanoparticle tracking analysis, and fluorescently labeled with Vybrant DiD for uptake experiments. Endocytosis inhibitors were used to determine dominant uptake pathways.

Results: Results indicate that in serous subtype (SKOV3 wt and SKOV3 cis), ChemoRC-EVs have higher uptake compared to SensC-EVs. Among the serous subtype inhibition of EV uptake by dynasore, nystatin and NAV2729 indicates that uptake into these cells is predominantly

through clathrin-mediated and caveolin-mediated endocytosis with minimal receptor-mediated involvement. Interestingly, SKOV3 wt cells internalized more ChemoRC-EVs than SensC-EVs, potentially contributing to chemoresistance.

In contrast, in the endometroid subtype, A2780 wt cells were observed to have higher tropism towards SensC-EVs than ChemoRC-EVs, whereas the cisplatin resistant cell line exhibits similar uptake levels by both types. In endometrioid subtype, uptake inhibition suggests that similarly to the serous subtype clathrin-mediated and caveolin-mediated endocytosis pathways are the main mechanism of uptake, whereas inhibition by nystatin in A2780 cis cells indicates uptake predominantly occurs through caveolin-mediated endocytosis.

Conclusions: These findings suggest that EV uptake mechanisms, and their tropism, vary by OC subtype, resistance status, and EV source. Generally, sensitive cells internalized more ChemoRC-EVs, implicating a potential role in the transfer of resistance traits.

Building on these findings, future work will explore therapeutic strategies that exploit these pathways. Specifically, EVs will be engineered to deliver anticancer agents directly into ovarian cancer cells, hijacking their uptake mechanisms to overcome chemoresistance. This approach could transform EVs from mediators of resistance into vehicles for therapeutic delivery, offering novel avenues for combating ovarian cancer progression and improving patient outcomes.

Word Count: 455

Keywords: Ovarian cancer, Extracellular vesicles, chemotherapy resistance

#58

Leukocyte-based Biomimetic Nanoparticles as siRNA Delivery in Triple-Negative Breast Cancer

Manuela Sushnitha, PhD [1,2,3], Chiara Mancino, PhD [1,2], Giulia Zaffaroni [1,2,4], Jenny Chang, MD [5], Assaf Zinger, PhD [1,2], and Francesca Taraballi, PhD [1,2]

[1] Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, Houston, TX; [2] Orthopedics and Sports Medicine, Houston Methodist Hospital, Houston, TX; [3] Department of Bioengineering, Rice University, Houston, TX; [4] Department of Biomedical Engineering, University of Houston, Houston, TX; [5] Houston Methodist Cancer Center, Houston, TX.

Keywords: siRNA, TNBC, Leukosomes, Inflammation, JAK/STAT3

Introduction: Compared to other breast cancers, triple-negative breast cancer (TNBC) is an aggressive sub-type characterized by lower 5-year survival rates and no targeted therapy[1]. Given the high inflammatory environment of these tumors, inflammation can serve as a targeting feature for the selective delivery of nanoparticles to the tumor site[2]. Upon reaching the tumor, these drug-loaded nanoparticles can inhibit key pathways implicated in fueling tumor growth and survival. JAK/STAT3 pathway, this pathway has not only been found to be overactive in TNBC, but also a driver of the high metastatic potential of this disease.

Hypothesis: Driven by the natural tropism of leukocytes to sites of inflammation, we developed a novel biomimetic nanoparticle, the Leukosome[3], that we loaded with STAT3 siRNA. Using this two-pronged approach, we hypothesize that integration of the proteins of leukocytes onto these particles enables them to target the tumor site, while the loading of siRNA will selectively inhibit the JAK/STAT3 pathway.

Methods: Leukosomes were synthesized using phospholipids, cholesterol and the extracted membrane proteins from leukocytes. Dynamic light scattering and zeta potential were used to determine the size, polydispersity and surface charge of the particles. Protein presence of the particles was verified using flow cytometry and SDS-Page, while Ribogreen assay quantified siRNA encapsulation. TNBC cell lines (4T1 and MDA-MB-231) were used to determine the uptake, internalization and the toxicity of the particles. Effective siRNA silencing was quantified by RT-PCR in 2D and 3D *in vitro* system. Lastly, nanoparticles targeting was studied using a murine TNBC model where mice were treated with labeled particles, organs collected and imaged using IVIS.

Results: Leukosomes were found to be roughly 120nm in size with a PDI less than 0.15 and negative surface charge. Flow cytometry and SDS-page gels confirmed the presence of proteins on the particles while achieving a 50% encapsulation efficiency of siRNA. Imaging confirmed the internalization of the particles in tumor spheroids while achieving 80% and 50% reduction in STAT3 expression 24 hours after treatment in 2D and 3D *in vitro* system respectively. *In vivo*, Leukosomes demonstrated exhibited a 2-fold increase in tumor accumulation with decreased clearance to the liver and spleen after 6 hours.

Conclusions: These results offer a new targeted drug delivery approach for TNBC where we can utilize the inflammatory conditions within the tumor as a targeting mechanism to

increase accumulation, while selectively inhibiting the JAK/STAT3 pathway only in tumor cells. Future work with this platform aims to study the *in vivo* effects of this two-prong approach in understanding the long-term therapeutic efficacy of siRNA-loaded Leukosomes in TNBC tumors, such as the ability to reduce the cancer stem cell niche and reduce metastasis.

Acknowledgements: This work was financially supported by the NIH (R56), Ruth Kirschcstein NRSA (F31), William Randolph Hearst Foundation and Cancer Prevention & Research Institute of Texas (Award ID RP170466).

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#59

Dense DNA Functionalization of Heme-Containing Proteins Enhances Peroxidase Like Activity

Neha Satish[1], Seungheon Lee[1], Yuanjie Sun[1], Atri Bhattacharya[1], and Devleena Samanta[1]

[1]Department of Chemistry, The University of Texas at Austin, Austin, TX 78712

Keywords: Enzyme Enhancement, Protein Spherical Nucleic Acid, Heme Proteins, Catalysis, Antimicrobial Effects

Proteins are often chemically modified with polymers, glycans, or synthetic moieties such as PEG to improve their stability, solubility, or pharmacokinetics.[1] While these strategies are effective at enhancing stability or circulation, these modifications frequently reduce enzymatic activity by sterically hindering substrate access or disrupting the active site environment.[2] For example, PEGylation has been shown to decrease the activity of several enzymes such as *l-N-carbamoylase* and lysozyme and modifications to glycosylation patterns can disrupt protease turnover and substrate recognition.[3,4] This trade-off is particularly critical for heme-containing proteins, a class of biomolecules with diverse functions ranging from antimicrobial defense (lactoperoxidase, LPO) to electron transport (cytochrome c) and oxygen binding (hemoglobin, myoglobin). Strategies that simultaneously stabilize these proteins while enhancing activity remain limited. To address this challenge, we explored dense DNA functionalization of heme-containing proteins to activate catalysis while preserving protein structure and function. We hypothesized that dense conjugation of short DNA strands onto heme-containing proteins, forming protein spherical nucleic acids (ProSNAs), would alter protein conformations in a manner that enhances access to the heme catalytic center, thereby improving peroxidase-like activity.

To test this hypothesis, lactoperoxidase, cytochrome c, hemoglobin, and myoglobin were chemically conjugated with short DNA oligonucleotides, yielding ProSNAs. UV-Vis spectroscopy was employed to confirm retention of the heme Soret band and quantify DNA loading. Gel electrophoresis verified successful conjugation. Enzymatic activity was assessed using the standard 3,3′,5,5′-tetramethylbenzidine (TMB)/H_2O_2 assay. Kinetic parameters (Km, Vmax, kcat/Km) were derived from Michaelis-Menten analyses. Circular dichroism spectroscopy was performed to probe conformational changes induced by DNA functionalization. Antimicrobial efficacy was examined through optical density measurements (OD600), colony forming unit (CFU) reduction assays, biofilm inhibition assays, and scanning electron microscopy (SEM) imaging of bacterial morphology.

DNA functionalization enhanced peroxidase like activity in all four heme-containing proteins compared to their native counterparts. The enhancement was most pronounced for lactoperoxidase and cytochrome c. Michaelis-Menten analyses revealed up to a threefold increase in catalytic efficiency for LPO SNA, attributed to an increased turnover rate and mildly improved substrate affinity. Circular dichroism measurements indicated

DNA modification driven conformational relaxation that exposed the heme site and facilitated substrate access. Systematic evaluation of DNA strand length revealed minimal influence, whereas higher DNA loading density correlated with greater catalytic enhancement, underscoring the importance of dense local DNA environments.

Antimicrobial studies demonstrated that LPO SNA significantly outperformed unmodified LPO in reducing MRSA and multidrug-resistant *E. coli* viability. CFU assays confirmed substantial bacterial killing, while biofilm inhibition experiments revealed enhanced suppression of biofilm growth. SEM imaging provided mechanistic evidence of extensive bacterial cell wall and membrane disruption following LPO SNA treatment, consistent with enhanced production of antimicrobial oxidants.

Taken together, these findings establish DNA functionalization as a broadly applicable approach for activating heme proteins and highlight its potential for developing protein-based antimicrobial therapies.

Acknowledgements
This work was supported by the National Science Foundation (CHE-2404334), the Packard Foundation (2024-77398) and the Welch Foundation (F-2209-20240404).

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#60

Intravascular Tissue Factor-Targeted Peptide Amphiphile Nanofibers for Non-Compressible Torso Hemorrhage

Trevor Stone [1], Sophia Maragos [2] Hai Xu [2], David Gillis [2], Lu Yu [2], Mark Karver [3] Timothy Pritts [4], Liam Palmer [5], Nick Tsihlis [1,2], Samuel Stupp [1, 3, 5], Melina Kibbe [1,6]

[1] Department of Biomedical Engineering, University of Virginia; [2] Department of Surgery, University of Virginia; [3] Center for Regenerative Medicine and the Department of Medicine, Northwestern University; [4] Department of Surgery, University of Cincinnati; [5] Department of Chemistry, Northwestern University; [6] Department of Cardiothoracic & Vascular Surgery, UTHealth Houston

Keywords: Peptide Amphiphile, Hemostasis, Pre-hospital Intervention

Introduction

Non-compressible torso hemorrhage is the leading cause of potentially preventable death for military and civilian trauma.[1] There exists a need to develop an intravascular treatment for non-compressible torso hemorrhage that is shelf-stable and effectively treats internal injury. Previous research in our lab has shown that nanofibers with FVII-derived targeting epitopes can bind to tissue factor (TF) expressed at the site of injury.[2,3] We modified tranexamic acid (TXA) with a tyrosine-8-quinolone (Y8Q) moiety and appended this to a peptide amphiphile (PA), creating a novel antifibrinolytic therapeutic designed to inhibit plasmin activity.

Hypothesis

We hypothesize that nanofibers bearing FVII-derived peptide sequences will target tissue factor (TF) at sites of injury and deliver antifibrinolytic Y8Q to bolster clot strength.

Methods

Targeted therapeutic nanofibers containing 5% of the TF-targeted PA with 5%, 10%, or 20% of the Y8Q PA (with the remainder being diluent backbone Ac-PEG_8-EEAAVV-K(C_{12}) PA) were co-assembled and tested. Transmission electron microscopy (TEM) images were collected for all co-assemblies. In vivo efficacy experiments were performed using a validated rat liver punch biopsy model of severe hemorrhage in male Sprague Dawley rats (250-350 g, n=6 per group). Hemodynamics were monitored invasively and blood loss was measured using pre-weighed gauze.

Results

Nanofiber structure was observed by TEM, confirming that the PA co-assemblies were self-assembling. Within the first 2 minutes post-injury, there was no significant difference in blood loss between the treatment groups. After the first 2 minutes post-injury, there was significantly less total blood loss for rats that received the 5%, 10%, and 20% Y8Q therapeutic nanofiber (5.2%, 4.9%, and 3.8% total blood volume lost, respectively) vs. rats that received saline (10.2% total blood volume lost; $p \leq 0.01$; n=4-5), with a 63% reduction noted for the 20% Y8Q group ($p \leq 0.001$). There was no difference in blood pressure between the therapeutic and control groups.

Conclusion

TF-targeted therapeutic nanofibers resulted in a significant reduction in blood loss with no difference in blood pressure. These data suggest that TF-targeted nanofibers delivering Y8Q hold great promise as a novel therapeutic to slow or stop hemorrhage. Future work will include nanofiber optimization for greater efficacy, assessing long-term safety, and evaluating additional therapeutics.

Acknowledgements

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#61

Photoacoustic Imaging of In Vivo Stability and Tumor Uptake of J-Aggregated ICG–Loaded Polymersomes in a Murine Model

Authorship: Claire E Jones[1], Ananthakrishnan Soundaram Jeevarathinam[1], **Paula Aguilera Rodriguez[1]**, Jongyeong Jeon[2], Mohammad Kawelah[2], Alexander Marras[2], Keith Johnston[2], Thanh Chung Vu, Yunfei Wen, Anil Sood, Konstantin Sokolov[1]*, and Richard Bouchard[1]*

[1] Department of Imaging Physics, MD Anderson Cancer Center, Houston, Texas 77030, United States.

[2] McKetta Department of Chemical Engineering, The University of Texas at Austin, Austin, Texas 78712, United States.

Division of Surgery, Department of Gynecologic Oncology and Reproductive Medicine

* RRBouchard@mdanderson.org and ksokolov@mdanderson.org

Introduction: Several aggressive cancer subtypes require antibody-based therapies guided by molecular assessment, but reliance on invasive biopsies limits noninvasive, longitudinal monitoring. Photoacoustic imaging (PAI) offers high spatiotemporal resolution with anatomical, molecular, and functional (e.g., SO_2) data. We previously used PAI to assess intratumoral folate receptor alpha (FRα)-targeted, liposome-encapsulated J-aggregated indocyanine green (JICG) in a murine ovarian cancer model [1]. Here, we introduce polymersome-encapsulated JICG (Ps-JICG; ~80 nm, PLGA-b-PEG) to improve circulation and tumor uptake. Polymersome encapsulation retains JICG at ~890 nm, enabling simultaneous PAI of SO_2 and probe signal unmixing [2]. Upon de-aggregation or leakage, ICG blueshifts to ~790 nm with increased fluorescence detectable by cryofluorescence tomography (CFT). We demonstrate Ps-JICG stability in naïve mice and Frα-targeted uptake in an ovarian cancer model using longitudinal PAI with complementary CFT.

Hypothesis: We hypothesize that polymersome-encapsulated J-aggregated ICG will establish a stable and distinctive platform for noninvasive, longitudinal photoacoustic imaging of tumor delivery and uptake.

Methods: Molecularly targeted Frα-Ps-JICG was synthesized, as previously described [2], and conjugated with anti-Frα antibodies. Volumetric, multiwavelength PAI was performed in regions encompassing the liver and spleen in athymic NCr mice (Taconic Biosciences) using the MSOT inVision 256-TF system (iThera). Imaging was performed pre-injection and repeated for 1hr following Frα-Ps-JICG (100 OD) IV injection. Additional PAI was performed at 24, 72, and 144hr post-injection. Fluence-corrected images were unmixed for oxy-/deoxyhemoglobin (i.e., SO_2) and Ps-JICG to estimate relative chromophore concentrations within liver and spleen VOIs. At assigned endpoints, mice were imaged with CFT on the Xerra (Emit Imaging) at 1, 72, and 144hr post-injection for ICG localization from JICG deaggregation. ICG fluorescence was analyzed within VOIs in the liver, spleen, gallbladder, and fecal matter. Additionally, molecular targeting using Frα-Ps-JICG was assessed in SKOV3-IP1 orthotopic ovarian tumor in athymic mice. Multi-wavelength, volumetric PAI was performed pre-injection and repeated for 1hr following Frα-Ps-JICG injection; tumor data were unmixed for JICG and SO_2.

Results: Longitudinal PAI imaging of liver and spleen showed Frα-Ps-JICG signal increased until 30 min post-injection in normal mice.For both organs, Ps-JICG signal significantly decreased by 24hr post-injection, suggesting clearance. In the spleen, Ps-JICG signal continue to decrease progressively until 144hr. ICG fluorescence measured with CFT remained low in the liver and spleen through 72hr post-injection, suggesting stability of remaining Ps-JICG (i.e., encapsulation and JICGs still intact). However, at 144-hr end-point, both spleen and liver had increased fluorescent signal, indicating compromised encapsulation and/or leakage of monomeric ICG. PA-imaging-based enhancement of accumulation of Frα-Ps-JICG was observed at 30min post-injection, suggesting tumor specificity after agent washout.

Conclusion: Longitudinal PAI presents a higher Frα-Ps-JICG signal in the liver compared to the spleen 24-144hr post-injection, suggesting clearance by the hepatic system. CFT results from liver and spleen show increased fluorescence (i.e., ICG) at 144hr post-injection indicating, a progressive breakdown of Ps-JICG's polymersome encapsulation and retention for at least 6days. PA tumor enhancement at early time-points (30min post-injection) in $Fr\alpha^+$ tumors demonstrate molecularly specific uptake into targeted cells. Further studies will evaluate tumor-site accumulation and preclinical biodistribution in tumor-bearing subjects to address safety concerns for future clinical translation.

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#63

Nanofluidic Electrochemical Pump for Wireless, On-Demand Tunable Drug Delivery

Marco M. Paci,[1,2], Nicola Di Trani,[1], Paolo Bolla,[1,3], Fabiana Del Bono,[1,3], Takuma Yoshikawa,[4,5], Isaac Tichy,[1,6], Patrick S. Stayton,[4,5], Alessandro Grattoni,[1,7,8]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA;
[2]College of Engineering, Swansea University, Swansea, SA1 8EN, UK;
[3]Department of Applied Science and Technology, Politecnico di Torino, Torino, 10129, Italy;
[4]Department of Bioengineering, University of Washington, Seattle, WA 98195, USA
[5]Department of Molecular Engineering & Sciences Institute, University of Washington, Seattle, WA 98105, USA;
[6]College of Engineering, University of Florida, Gainesville, FL 32603, USA;
[7]Department of Surgery, Houston Methodist Research Institute, Houston, TX 77030, USA;
[8]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA.

Keywords
Nanofluidics; Electrochemical pump; Implantable drug delivery; Remote control; Precision medicine

Abstract
Chronic diseases require long-term pharmacotherapy, yet conventional oral and injectable routes are limited by fluctuating plasma drug levels, poor adherence, and systemic side effects. Implantable drug delivery systems offer sustained release, but most rely on passive mechanisms lacking programmable or adaptive control[1]. To address this need, we developed a nanofluidic membrane-based electrochemical pump that combines the simplicity, reproducibility and responsiveness of electrochemical pressure generation with the precision of nanofluidic flow control[2]. We hypothesized that in situ electrochemical gas generation within a sealed reservoir could drive pressure-mediated convective transport across nanochannels, achieving tunable, reversible, and charge-independent drug release suitable for integration into implantable closed-loop therapeutic systems. Nanofluidic membranes (200–270 nm channels, Ti/Pt-coated) were fabricated using silicon micro/nanofabrication and sputtering, and electrochemical characterization (CV, EIS, chronoamperometry) was performed under physiological conditions. Pressure buildup under alternated -2 VDC bias (ON state) and open circuit potential (OFF state) was monitored in a custom dual-reservoir system with integrated sensors. Finally, in vitro release studies were performed with model drugs, such as Vitamin B12, cabotegravir, and polystyrene sulfonate to evaluate the system performance. The wireless operation of the platform was demonstrated with a Bluetooth-enabled PCB powered by a coin-cell battery, and the application of –2 VDC produced stable hydrogen evolution, yielding reservoir pressures of 7.06 ± 2.56 mbar and actuation currents of 2.31 ± 0.36 mA at 4.62 ± 0.43 mW power. Reversible ON–OFF cycling demonstrated precise pressure modulation. Drug release rates were tunable within 1–10 µg/h across diverse compounds, independent of molecular charge. In conclusion, this nanofluidic electrochemical pump enables low-power and wireless control of drug release at therapeutically relevant rates. Its scalability, molecular versatility, and compatibility with implantable electronics

highlight strong potential for integration into next-generation, closed-loop precision medicine platforms.

Acknowledgements
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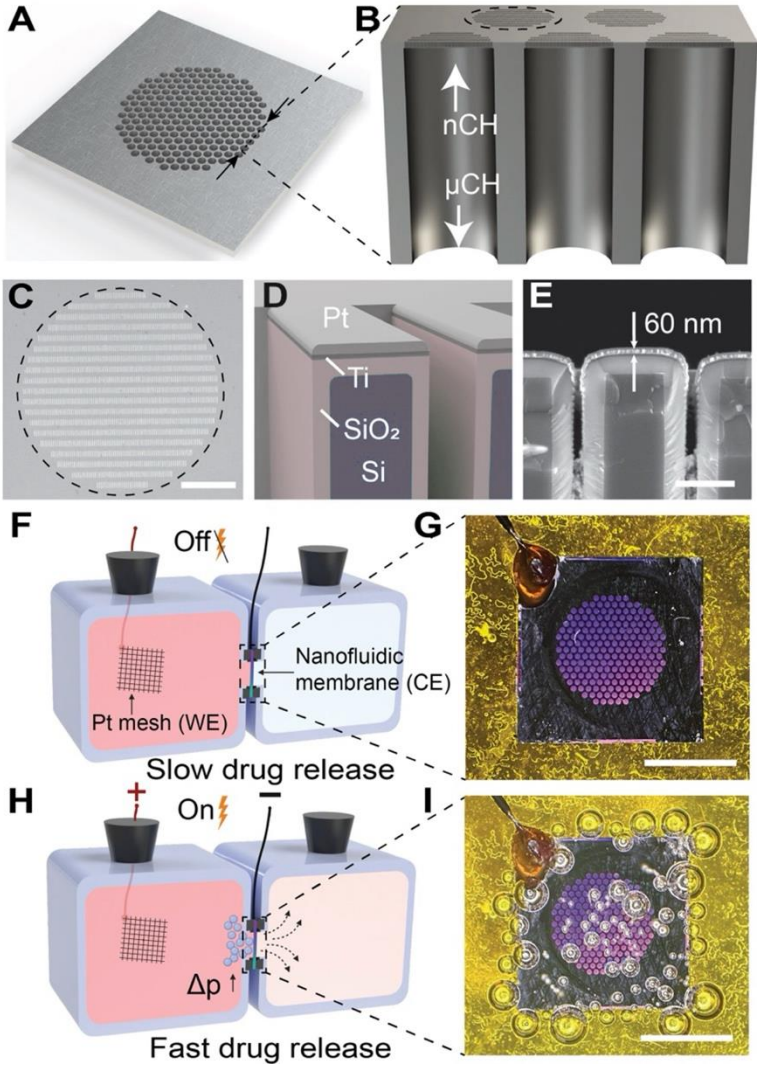

#64

Leveraging conditionally activatable fusogenic lipid nanocarriers for precision cancer immunotherapy

Qian Zhong
Division of Molecular Pharmaceutics and Drug Delivery , College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, USA

Introduction: Macromolecular drugs, such as peptides, enzymes, intrabodies and ribonucleoprotein particles (RNPs), are currently in development. These tools are designed to act directly on intracellular targets for applications ranging from disrupting metabolic abnormalities, to regulating signaling pathways, to immune response modulation, as well as gene editing. However, on-target drug delivery to pathological environments and subsequent internalization by abnormal cells – either via translocation across the plasma membrane, or via endosomal escape – remain long-standing physiological and subcellular challenges that limit therapeutic efficacy. To address these issues, we harnessed the catalytic activity of proteases present at lesion sites to engineer a class of conditional fusogenic liposomes (C-FLIPs) to enable efficient cytosolic delivery of amnio acid-based biomacromolecules (e.g., peptides, proteins). Our hypothesis is that C-FLIPs fuse with target cells only after catalytic reactions performed by disease-dysregulated proteases successfully remove fusion inhibitors. This fusion reaction at the cell membrane subsequently releases impermeable payloads directly into cytoplasm, thus bypassing endocytic pathways in which peptide bonds are susceptible to enzymatic degradation (Fig. 1). In contrast, C-FLIP fusogenicity is silenced in normal tissues and cells due to the absence of the target protease activity.[1]

Materials and Methods: C-FLIPs are comprised of 3 major components – fusion inhibitors, disease-specific protease substrates, and drug-loaded fusogenic liposomes (FLIPs). The C-FLIPs were conjugated with pre-designed fusion inhibitors via protease-activatable substrates. The C-FLIPs were subsequently optimized for substrate cleavage kinetics, for fusion with various cell types, and for delivering different peptide/protein-based biologics. We then leveraged the C-FLIPs to deliver granzyme B, termed "synthetic killer cells", to assess their antitumor efficacy in two immunologically cold tumor models – lung and colorectal cancer. Synergistic effects of synthetic killer cells with immune checkpoint blockade (ICB) and/or RNAi therapeutics were also evaluated in the same cancer models. Finally, we sought to understand the crosstalk between synthetic killer cells and immune microenvironment in tumors.

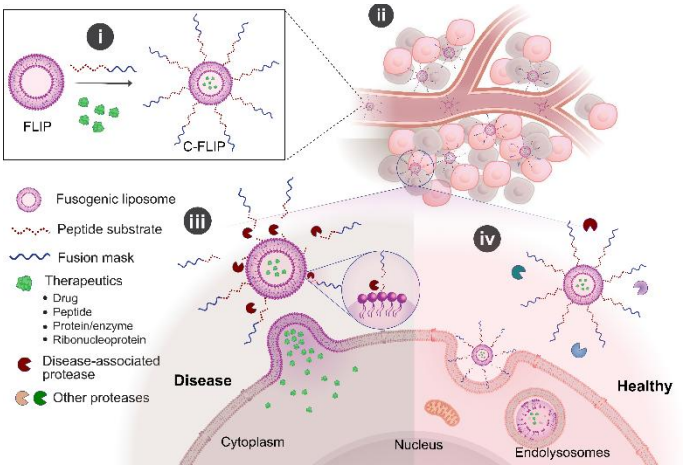

Figure 1. Schematic showing cytosolic delivery of macromolecular drugs via C-FLIP at the target site. C-FLIPs are conjugated on the surface with protease substrate-linked fusion inhibitors. At disease sites of interest, upregulated proteases quickly remove fusion inhibitors by cleaving the substrate linkers. Shedding of the inhibitors allows the liposomes to fuse with cell membranes for direct release of the carried cargos into the cytosols – bypassing the endocytic pathways. In contrast, the abundant fusion inhibitors remain on the surface due to lack of aberrant proteolytic activities present in off-target, healthy organs and dramatically suppress fusogenicity of the C-FLIPs. Consequently, they are subsequently taken up predominantly via endocytosis by healthy cells, leading to functional quiescence of the cargos.

Results and discussion: our C-FLIPs have demonstrated in vivo functional capacity to fuse with various cells, and to deliver cytotoxic proteins that induce cell death and peptide-based proteolysis-targeting chimera (PROTAC) to promote targeted protein degradation. Building on the optimal C-FLIP formulation, we developed targeted synthetic killer cells that release granzyme family proteins into malignant cells in a protease-activatable, pore-forming motif-free (e.g. perforin) manner. Synthetic killer cells via intravenous administration significantly suppressed tumor growth in mouse lung and colorectal tumor models by triggering gasdermin E-mediated pyroptosis, and increased infiltration of CD8+ effector T cells. Furthermore, ICB and/or silencing of intracellular serine protease inhibitors strongly synergized with the therapeutic impact of synthetic killer cells, and even eradicated tumors in some of the treated animals. The combination of synthetic killer cells and ICB also promotes the expansion of resident CD8+ memory T cells in the lungs, which prolongs improved protection against tumor recurrence and metastasis.

Conclusion: Therefore, the non-viral, cell-free C-FLIPs represent a promising platform that addresses both imprecise delivery to target sites and inefficiencies in the cytosolic delivery of impermeable biomacromolecules, and could enable new opportunities in tackling intracellular delivery of a large palette of macromolecular therapeutics for immunotherapy and gene editing.

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#65

Bioenergetic reprogramming for attenuation of mesenchymal stem cell senescence

Hyunho Lee, Matteo Massaro, Gherardo Baudo, Pinar Atalay, Elvin Blanco

Department of Nanomedicine
Houston Methodist Academic Institute

Introduction: Cellular senescence is defined as a state of irreversible cell cycle arrest induced by oxidative stress and the passage of time, and is accompanied by a decline in energy metabolism, antioxidant capacity, and regenerative potential. In stem cell therapeutics, senescence is increasingly recognized as a major determinant of therapeutic efficacy. Mesenchymal stem cells (MSCs), although widely studied for their regenerative potential, are prone to senescence due to oxidative stress, mitochondrial dysfunction, and repeated *in vitro* expansion. This progressive loss of proliferative capacity, metabolic balance, and stemness undermines their therapeutic capabilities. Thus, strategies that reinforce mitochondrial health to attenuate senescence may be pivotal for sustaining MSC efficacy.

Hypothesis: We hypothesized that induction of nuclear respiratory factor 1 (NRF1), a master regulator of mitochondrial biogenesis, could metabolically reprogram MSCs to suppress senescence by preserving mitochondrial function.

Methods: Human bone marrow-derived MSCs (BM-MSCs) were transfected with NRF1 mRNA or scrambled (SCR) mRNA control. NRF1 induction was confirmed by scRNA-seq, qRT-PCR, and western blotting for NRF1, TFAM, and COXIV. Mitochondrial mass, function, and dynamics were assessed using confocal microscopy, flow cytometry (MitoTracker), western blotting, citrate synthase activity, and Seahorse analysis of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Oxidative stress–induced (H_2O_2, 250 μM, 1 h) and replicative (passage 10, p10) senescence models were employed. Senescence was evaluated by SA-β-gal staining and expression of p53 and p16, while ROS was measured by MitoSOX (mitochondrial ROS) and H_2DCFDA (intracellular ROS). Mitochondrial membrane potential was assessed using JC-1, and metabolic status by ATP production and lactate quantification. Comparative groups included the senolytic ABT263, pharmacologic inducers of mitochondria biogenesis (bezafibrate), and mitochondria biogenesis inhibitor (doxycycline).

Results: NRF1 induction in BM-MSCs triggered metabolic and functional reprogramming. NRF1 mRNA transfection drove robust expression of NRF1 and downstream regulators TFAM and COXIV, resulting in greater mitochondrial mass, elevated mtDNA copy number, and enhanced citrate synthase activity. These changes preserved mitochondrial integrity under oxidative and replicative stress, as shown by reduced ROS accumulation, maintenance of membrane potential, and restoration of bioenergetics. Single-cell transcriptomics revealed increased OXPHOS, with suppression of glycolysis and senescence-related gene expression. Notably, NRF1-primed MSCs exhibited diminished senescence under both stress models, evidenced by reduced SA-β-gal activity and downregulation of p53, p21, and p16, indicating strong resistance to senescence. Importantly, NRF1 induction outperformed pharmacological inducers of mitochondrial biogenesis and achieved effects comparable to or exceeding the senolytic ABT263, underscoring its translational potential to sustain MSC therapeutic efficacy.

Conclusion: NRF1 induction reprogramed BM-MSC metabolism toward OXPHOS dominant energy production, attenuated both stress-induced senescence and preserved regenerative potential by enhancing mitochondrial biogenesis. NRF1 also elevated mitochondrial antioxidant enzymes, counteracting oxidative stress in both premature and replicative senescence models. Notably, NRF1 exerted global suppression of senescence-associated markers, demonstrating effects superior to the senolytic agent ABT263. These findings position NRF1-based metabolic priming as a promising strategy to enhance the durability and therapeutic success of MSC-based interventions.

Key words: Nuclear respiratory factor 1 (NRF1), mesenchymal stem cells (MSCs); cell senescence; metabolic potentiation; oxidative stress

#66

Mitochondrial Replenishment Restores Neuronal Function in Alzheimer's Disease

Gherardo Baudo[a], Matteo Massaro[a], Hyunho Lee[a], Haoran Liu[a], Elvin Blanco[a,b,c]

[a]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, 77030, USA
[b]Department of Medicine, Weill Cornell Medical College, New York, NY, 10065, USA
[c]Department of Cardiology, Houston Methodist DeBakey Heart and Vascular Center, Houston Methodist Hospital, Houston, TX, 77030, USA

Keywords
Alzheimer's disease; mitochondrial replenishment; amyloid-β; neuronal bioenergetics; neuroprotection

Background

Alzheimer's disease (AD) is the most prevalent form of dementia, affecting more than 7 million Americans, with cases projected to triple worldwide by 2050. The economic burden is immense, with current US health and long-term care costs approaching $384 billion annually for individuals with AD and other dementias projected to exceed $1 trillion by 2050[1]. While amyloid-β (Aβ) remains a hallmark of AD pathogenesis and a major therapeutic target, current Aβ-directed treatments face important limitations, principally that they provide only partial efficacy once clinical symptoms emerge. This underscores the need for complementary strategies addressing additional mechanisms driving disease progression. Among these, mitochondrial dysfunction—marked by impaired oxidative phosphorylation (OXPHOS), excessive reactive oxygen species (ROS), abnormal mitochondrial dynamics, and defective mitophagy—represents a critical nexus of neuronal injury. Restoring mitochondrial function offers the potential to preserve synaptic integrity and counteract neurodegeneration[2].

Hypothesis

We hypothesized that transplantation of exogenous mitochondria functionalized with a dextran–triphenylphosphonium conjugate (Dex-TPP/Mt) into amyloid-β (Aβ)-exposed neurons would restore mitochondrial function, counteract bioenergetic failure, and attenuate neurodegenerative processes.

Methods

Mitochondria were isolated from murine livers, functionalized with Dex-TPP[3], and transplanted into SH-SY5Y neuronal cells stimulated with Aβ (2 μM). Mitochondrial function was assessed by confocal microscopy, flow cytometry, and Seahorse metabolic profiling. Western blot was used to evaluate proteins regulating mitochondrial dynamics (Drp1, Fis1, MFN2, OPA1), mitophagy (PINK1, Parkin, LC3), apoptosis (Bax, Bcl-2, caspase-3, pJNK), and glycolytic shift (HIF-1α, HKII, PKM2). Neurite length was quantified to assess synaptic architecture.

Results

Dex-TPP/Mt significantly reduced Aβ-induced mitochondrial ROS, preserved membrane potential, and restored dynamics by lowering Drp1/Fis1 while upregulating MFN2/OPA1. Aberrant mitophagy activation (PINK1, Parkin, LC3) was normalized, consistent with improved mitochondrial quality. Metabolically, Dex-TPP/Mt reversed the Aβ-driven glycolytic shift: oxygen consumption rate increased, extracellular acidification decreased, and ATP production rose,

indicating restored reliance on OXPHOS. Apoptotic pathways were attenuated, as evidenced by reduced Bax/Bcl-2 ratio, decreased caspase-3 and pJNK, and fewer Annexin V-positive cells. Importantly, Dex-TPP/Mt preserved neurite length, highlighting protection of neuronal connectivity.

Conclusion

Mitochondrial replenishment through Dex-TPP/Mt confers broad neuroprotective effects by restoring bioenergetics, reducing oxidative stress, and safeguarding neuronal structure in Aβ-challenged neurons. This strategy addresses core drivers of AD progression, offering a mechanistically distinct approach complementary to existing amyloid-targeted therapies. Ongoing studies aim to validate these findings *in vivo* and accelerate translation toward clinical applications.

Acknowledgements

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#67

Reinforcing mitochondrial fitness as a strategy against $CD8^+$ T Cell exhaustion

Pinar Atalay, Matteo Massaro, Hyunho Lee, Gherardo Baudo, Junhua Mai, Elvin Blanco

Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, 77030

Keywords

Cancer; T-cell exhaustion; mitochondrial function; metabolic potentiation, adoptive cell therapies

Background

Metastatic breast cancer (MBC) remains largely incurable (5-year survival <30%; >40,000 U.S. deaths/year). Standard therapies offer modest benefits, and the efficacy of immune checkpoint blockade (ICB) and adoptive cell transfer (ACT) is limited by an immunosuppressive microenvironment and metabolically exhausted tumor-infiltrating $CD8^+$ T cells. Restoring T-cell mitochondrial fitness and oxidative metabolism is therefore critical to sustain persistence and cytotoxicity and improve immunotherapy outcomes in MBC.

Hypothesis

We hypothesized that overexpressing nuclear respiratory factor 1 (NRF-1), a master regulator of mitochondrial biogenesis, would reinforce mitochondrial function, reduce exhaustion, and enhance the survival and anti-tumor function of chronically stimulated OT-1 $CD8^+$ T cells.

Methods

$CD8^+$ OT-1 T cells (TCs), which are transgenic for an OVA-specific TCR, were isolated from the spleens of transgenic mice and activated *in vitro* for 7 days using anti-CD3/CD28 antibodies and IL-2 to model chronic stimulation and exhaustion. Cells were then transfected with NRF1 mRNA or scrambled controls. Mitochondrial biogenesis and content were assessed by western blot for NRF1, TFAM, and COX IV, together with MitoTracker intensity for mitochondrial mass. Mitochondrial dynamics were evaluated by expression of fusion proteins MFN2 and OPA1 and fission proteins DRP1 and Fis1. Mitochondrial health was measured using MitoSOX for mitochondrial ROS and JC-1 dye for membrane potential. Cellular metabolism was profiled through ATP quantification and by examining glycolytic regulators HKII, PFKFB3, and HIF-1α. Exhaustion was evaluated by flow cytometry for PD-1 and CTLA-4 co-expression, while apoptosis was assessed by western blot for Caspase-3 and Bax/Bcl-2. Functional assays included examination of IFN-γ and TNF-α cytokine production and cytotoxicity assay against E0771-OVA-Luc breast cancer cells using LDH-Glo.

Results

NRF1 overexpression improved mitochondrial fitness in exhausted OT-1 TCs. Expression of TFAM and COX IV increased, and mitochondrial mass was restored to levels similar to non-

exhausted controls. NRF1 induction decreased oxidative stress by reducing mitochondrial ROS accumulation and maintained mitochondrial health by preserving mitochondrial membrane potential in OT-1 TCs. Mitochondrial dynamics were normalized, with fusion proteins MFN2 and OPA1 restored, and fission proteins DRP1 and Fis1 reduced. Metabolically, exhausted T cells exhibited higher expression of glycolytic markers together with reduced ATP production. NRF1 suppressed glycolytic markers and restored high ATP levels. In parallel, NRF1 reduced the fraction of PD-1^{+}CTLA-4^{+} double-positive exhausted cells from ~22–24% to ~7%. Apoptosis was reduced, with decreased caspase-3 expression and a normalized Bax/Bcl-2 ratio. Functionally, NRF1 partially restored IFN-γ production, while TNF-α remained stable. Finally, cytotoxicity assays showed that NRF1-reinforced OT-1 cells mediated significantly greater tumor-cell killing of E0771-OVA-Luc cells, indicating that metabolic reinforcement translated to improved antitumor activity.

Conclusions

NRF1 reprogrammed exhausted OT-1 CD8^{+} T cells by restoring mitochondrial function. These shifts enhance effector function and tumor killing, supporting metabolic reinforcement as a strategy for effective ACT.

#69

Radiopaque Nanoparticle-Loaded, Bioresorbable Polymeric Perivascular Wrap Modulates Transcriptomic Pathways Associated with Arteriovenous Fistula Failure

Allan John R. Barcena [1], Marvin R. Bernardino [1], Archana Mishra [1], Dominic Karl M. Bolinas [1], Natalie Fowlkes [2], Richard R. Bouchard [3], Jizhong Cheng, MD [4], Steven Y. Huang [1], Marites P. Melancon [1]

[1] Department of Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030; [2] Department of Veterinary Medicine and Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030; [3] Department of Imaging Physics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030; [4] Division of Nephrology, Department of Medicine, Selzman Institute for Kidney Health, Baylor College of Medicine, Houston, TX 77030;

Introduction: Arteriovenous fistula (AVF) failure increases morbidity and mortality for patients relying on hemodialysis. To address this, we developed electrospun bioresorbable perivascular wraps that can provide mechanical support and prevent AVF failure. These wraps can also be infused with radiopaque nanoparticles and therapeutics for in vivo monitoring and enhanced efficacy, respectively. We have demonstrated in previous studies that the wraps can mitigate neointimal hyperplasia, the main culprit of AVF failure. However, the underlying molecular mechanisms for their therapeutic benefit remain largely unknown, hindering further optimization and clinical translation. Hence, we used transcriptomic analysis to elucidate how perivascular wraps prevent AVF failure.

Hypothesis: We hypothesize that the bioresorbable perivascular wraps, which provide mechanical support to the AVF, could reduce hemodynamic stress and metabolic demand. Furthermore, we expect that the wraps will elicit an immune response primarily driven by macrophages and that the addition of radiopaque nanoparticles will not significantly alter this response.

Methods: Radiopaque bismuth nanoparticles (BiNPs), synthesized in the laboratory through thermal decomposition with an average size of 3.29 ± 0.511 nm, and poly(ε-caprolactone) (PCL) were dissolved and electrospun to produce perivascular wraps. AVFs were created in Sprague-Dawley rats with surgically induced chronic kidney disease and assigned to one of three groups: control (no wrap), PCL wrap, or BiNP-loaded PCL (PCL-Bi) wrap. After 8 weeks, the AVFs were harvested for total RNA extraction and library preparation. RNA sequencing was performed using the Illumina platform. Gene expression was quantified, and differentially expressed genes were identified using the DESeq2 package with a significance threshold of $p < 0.05$. Subsequently, gene set enrichment analysis was conducted to identify key biological pathways altered by the perivascular wraps.

Results: Compared to untreated controls, both PCL and PCL-Bi exhibited a significant downregulation of blood circulation, oxidative phosphorylation, and muscle system processes.

This suggests the wraps mitigate neointimal hyperplasia by modulating hemodynamic stress, reducing local metabolic demand, and inhibiting smooth muscle cell proliferation. Both wraps also showed an upregulation of pathways associated with innate immune and inflammatory responses, including myeloid leukocyte activation and cytokine production, consistent with foreign body macrophage response to implanted devices. Compared to PCL, PCL-Bi showed an upregulation in detoxification and antioxidant pathways, likely attributable to the clearance of BiNPs. Although the majority of small (<6 nm) BiNPs are expected to be cleared renally, BiNPs can undergo oxidative decomposition under physiological conditions. PCL-Bi also showed downregulation of angiogenesis and adaptive immune response, suggesting a potential therapeutic benefit towards modulation of vascular remodeling and immune response.

Conclusion: This study provides the first molecular pathway-level proof of mechanism for the efficacy of radiopaque nanoparticle-loaded, bioresorbable polymeric perivascular wraps, which prevent AVF failure likely by modulating hemodynamic stress, reducing local metabolic demand, and inhibiting smooth muscle cell proliferation. Future studies using single-cell and spatial transcriptomics are needed to confirm the precise localization of these processes and to better understand how the local immune response to both the electrospun polymer and nanoparticles influence efficacy.

Keywords: bismuth nanoparticles, medical device, polycaprolactone, RNA sequencing, vascular access

Acknowledgements: This study was supported by National Heart, Lung, and Blood Institute (5R01HL159960), the American Heart Association (24TPA1280217, 25DIVSUP1477011), and the American Society of Diagnostic and Interventional Nephrology.

#70

Surface-Modified T cells to Engineer Immune Cell Infiltration through 3D Collagen

Zhenjiang Zhang*, Michael R. King
Department of Bioengineering, Rice University, Houston, TX 77030

Although CAR-T cell therapy has shown great success in hematological malignancies, their application to solid tumors has been hampered by the inability of the transferred T cells to infiltrate tumors. To reach tumor cells in a tumor, CAR-T cells must penetrate the extracellular matrix (ECM). Collagen is the most abundant protein in the ECM with significantly more collagen content in tumors than in healthy tissues, resulting in an increased collagen deposition around the tumor. Previous studies have shown that the dense collagen layer disrupts the tumor infiltration of immune cells. The turnover of collagen matrix is partly regulated by matrix metalloproteinases (MMPs) that are capable of proteolysis of ECM components. In this study, we demonstrated a liposome fusion technology to anchor recombinant MMP-8 in the membrane of Jurkat and primary human T cells that guide their way through a dense collagen layer by the degrading matrix protein.

Liposomes were prepared using the hydration/extrusion method. Human immortalized Jurkat T cells were used for liposome fusion with the cell membrane. After fusion, the cells were analyzed by confocal microscopy and flow cytometry. For the transmigration assay, transwell inserts were coated with Type I collagen. All human blood samples were collected from fully consented donors. After the blood was treated with liposomes, primary T cells were isolated to test their fusion with liposomes and for subsequent migration studies as well.

We incorporated phase separation in fusogenic liposomes to reduce their cytotoxicity without compromising their fusogenicity (Figure A). The liposomes immediately fused with the membranes of Jurkat cells following the mixing of the two (Figure 1B). After the membrane fusion, the lipids of the liposomes stayed on the membrane without being detached or internalized for at least 48 hr (Figure 1B). Proteins conjugated on the liposomes, as represented by GFP, can be efficiently anchored in the membrane of Jurkat cells (Figure 1C). The fusogenic liposomes can also efficiently fuse with primary T cells isolated from human and mouse blood. In the transmigration assay, >45% more Jurkat cells modified with MMP-8 were able to migrate to the bottom well than those without MMP-8. The MMP-8 proteins added to Jurkat cells at least partly stayed on the cell membrane after the migration (Figure 1D). After staining with fluorescent antibody, ~10 μm pores were found on the collagen layers through which MMP-8-modified Jurkat cells just passed, suggesting collagen degradation occurred during the migration. More interestingly, the second batch of Jurkat cells can migrate through the collagen layers with ~10 pores much faster than fresh collagen layers. These results support the idea that MMP-8 anchored on cell membrane can facilitate cell transmigration through collagen by degrading the major ECM protein. A small population of leader cells with exogenous MMP-8 could be enough to promote infiltration of more extensive T cells. We demonstrated a novel liposome/plasma membrane fusion technology to add exogenous MMP-8 to the membrane of T cells. This technology could significantly improve the outcomes of a wide range of therapies based on viable cells.

#71

Title: Advancing Cell-Mediated Drug Delivery to Treat Venous-Thromboembolism

Veronica Lisi [1], Emily Barnet [1], Suhon Kim [1], Nancy Buechler [1], Erica Burham [2], Rachel Pollard [2], Edward Bahnson [3],Nathalie Pinkerton [2], Sophie Maiocchi [1]

[1] Department of Biomedical Engineering, Wake Forest University School of Medicine, Winston-Salem, NC, 27101, USA; [2] Department of Chemical and Biomolecular Engineering, Tandon School of Engineering, New York University, New York, NY, 11201, USA; [3] Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC, 27599, USA;

Keywords: Cell-mediated drug delivery, NETs-targeted therapy.

Introduction: Each year, nearly 1 million Americans are affected by deep vein thrombosis (DVT) with 10-30% of these patients dying within 1 month of diagnosis due to pulmonary embolism (PE). Systemic thrombolysis rapidly reduces thrombus burden, but substantially increases the risk of major bleeding, and potentially fatal intracerebral hemorrhage. Catheter directed thrombolysis (CDT) employs lower tPA doses, but bleeding complications remain, is invasive, and only accesses large proximal thrombi, excluding distal thrombi and microcirculation (~20 % of patients are eligible). There is an urgent need for an adjunct therapy to thrombolysis that enhances clot dissolution while minimizing bleeding, and for a non-invasive, systemically administered therapeutic approach that achieves targeted delivery of therapies to the clot, thereby minimizing off-target effects. Neutrophil Extracellular Traps (NETs), which are extracellular networks of DNA/protein expelled by activated neutrophils, are critical components of venous thrombi. NETs are highly pro-thrombotic and impair clot thrombolysis. NETs are not essential to hemostasis and thus NET degradation is not expected to promote bleeding. NET degradation is a promising therapeutic approach to enhance thrombolysis efficacy, while minimizing bleeding. DNase 1 protein, a safe, low-cost, FDA-approved therapy for cystic fibrosis, degrades NETs. Accordingly, DNase 1 enhances *ex vivo* thrombolysis of human clots by 20–60%. Our **primary objective** is to develop a delivery strategy to achieve thrombus penetration of the NET-degrading therapy: DNase 1. To achieve thrombus-targeting we will use cell-mediated drug delivery (CMDD), using Nϕ as delivery vehicles for DNase 1 packaged inside polymeric NP (DNP).

Hypothesis: Neutrophils-mediated delivery of DNP will result *in vivo* intra-thrombus delivery, DNase 1 release and NET degradation.

Methods: DNP are synthesized using inverse Flash Nanoprecipitation (iFNP). To evaluate CMDD efficiency, we employ a murine inferior vena cava (IVC) stenosis model of venous thrombosis, which reliably induces clot formation and NET accumulation. *Ex vivo*, fluorescently labelled mouse bone marrow-derived neutrophils are loaded with DNPs and assessed for their ability to target thrombi, allow DNP exocytosis on thrombi NETs and their degradation through the DNase1 action.

Results: We successfully synthesized DNP using iFNP with a DNase 1 loading efficiency of 1.1 ± 0.3%. DNP effectively degraded NETs *in vitro* and were efficiently internalized by

neutrophils *ex vivo*. Neutrophils show also DNP exocytosis under *in vitro* stimulation conditions (4h, 100 nM PMA), moreover, they successfully mediated the delivery of NPs to *in vitro* thrombi, demonstrating their potential as targeted drug carriers. In *in vivo* DVT murine model *ex vivo* Neutrophils retroorbitally injected showed a localization at the IVC-clot.

Conclusions: Encapsulating therapeutic proteins in polymeric NPs, combined with CMDD, represents a promising strategy to improve treatment of VTE by improving targeted drug delivery while reducing bleeding risks. Our *in vitro* and *in vivo* findings demonstrate that *ex vivo* neutrophils can infiltrate organs, making them strong candidates for transporting DNP to thrombi. These results highlight the potential of CMDD for targeted anti-thrombotic therapy, which could help overcome thrombolytic resistance and improve patient outcomes in VTE.

Acknowledgements: R00HL157690-04, NHLBI. Targeted drug delivery for the treatment of cardiovascular disease and its clinical complications

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#72

Title: **An Injectable Polymeric Hydrogel for Intramyocardial Delivery and Retention of Nanoparticles**

Antonio Martino PhD[1,2], Blake C. Fallon[1], Amber Lee Royal[1], Giorgio Brero MS[1,3], Rossana Terracciano PhD[1], Yareli Carcamo-Bahena[1] MS, Richard C. Willson PhD[4], Nilesh Mathuria MD[5], Carly S. Filgueira PhD[1,6]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
[2]Department of Material Science and Engineering, University of Houston, Houston, TX, USA
[3]Department of Mechanical and Aerospace Engineering, Polytechnic University of Turin, Turin, Italy
[4]Department of Biochemical & Biophysical Sciences, University of Houston, Houston, TX, USA
[5]Houston Methodist Heart and Vascular Center, Houston Methodist Academic Institute, Houston, TX, USA
[6]Department of Cardiovascular Surgery, Houston Methodist Research Institute, Houston, TX, USA

Keywords: Alginate hydrogel, Intramyocardial delivery, Cardiac retention, Gold nanoshells, Computed tomography

Introduction: Cardiac delivery faces persistent barriers that limit efficacy, particularly rapid systemic washout of therapeutic agents and poor retention at the target site.[1] These challenges are amplified for sensitive cargo, such as cells or nanoparticles, where high injection pressures and mechanical stress cause damage, poor localization, and/or clearance.[2] Traditional pre-formed hydrogels have shown limited success due to superficial placement, poor injectability, and inability to conform to an irregular shape in tissues. Injectable, *in situ* crosslinking hydrogels represent a promising myocardial delivery platform, enabling localized, minimally invasive administration with sustained tissue retention.[3] Alginate, a clinically approved biopolymer, undergoes rapid gelation when exposed to divalent cations such as calcium, offering a safe, tunable hydrogel system. By incorporating gold nanoshells (GNS), the hydrogel exhibits radiopacity, allowing real-time image-guided monitoring with fluoroscopy and computed tomography (CT).

Hypothesis: We hypothesize that an injectable calcium alginate hydrogel carrying GNS cargo can be formed *in situ* through simultaneous precursor injection. We further hypothesize that the system will demonstrate uniform particle encapsulation with prolonged myocardial retention *in vivo*.

Methods: We co-delivered a sodium alginate precursor (1% w/v) solution loaded with GNS alongside a calcium chloride crosslinker (3% w/v) solution. Upon co-injection, rapid ionic crosslinking yielded a GNS–alginate hydrogel within the myocardial tissue of domestic swine. For epicardial delivery, open-chest intramyocardial injections (n=5 sites) were performed under direct visualization. For endocardial delivery, closed-chest intramyocardial injection was achieved into the left ventricular wall (n=3 sites) under fluoroscopic guidance. Hydrogel formation, volume, and radiopacity were monitored for 3 hours post-injection using serial CT and 3D reconstructions. Gross pathology of explanted tissue was used as method to confirm localization and GNS retention.

Results: CT imaging demonstrated robust *in situ* hydrogel formation at all target sites, with consistent radiopacity confirming nanoparticle encapsulation. Epicardial 1 mL precursor injections produced localized GNS-gel deposits averaging 80.04 ± 24.89 mm^3 with stable attenuation (229.66 ± 36.19 HU). Endocardial delivery inclusive of Omnipaque350 as the precursor solvent yielded larger volumes (729.33

± 293.03 mm³) and stronger attenuation (599.33 ± 112.49 HU), permissive of deeper myocardial penetration. Both approaches showed excellent spatial stability, with no evidence of migration, nanoparticle washout, or adverse events during the 3-hour study. Gross pathology confirmed localized GNS retention with intact hydrogel formation.

Conclusion: This study demonstrates the feasibility of an injectable, *in situ* crosslinking calcium alginate hydrogel for cargo delivery and retention in dynamic cardiac tissue. Epicardial delivery, though more invasive, permitted precise control, while endocardial delivery provided a minimally invasive, clinically translatable option. Both routes yielded stable, real-time hydrogel formation, sustained radiopacity, and robust retention over an image-guided acute 3-hour porcine study. These findings highlight the potential of this hydrogel system to overcome rapid washout and poor retention in cardiac therapy. By combining biocompatibility, injectability, and image-guided monitoring, this platform represents a versatile strategy for advancing localized cardiovascular nanomedicine.

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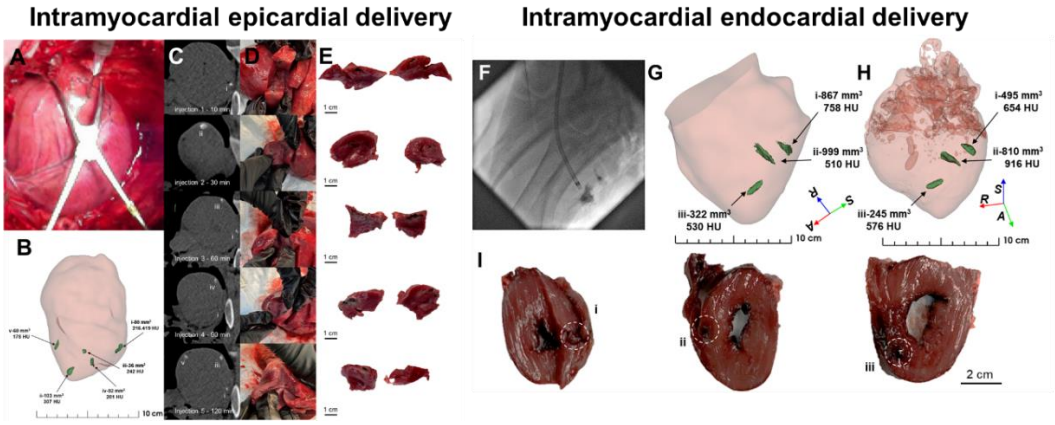

Figure 1. (A) Epicardial delivery: direct injection of GNS–alginate hydrogel into the beating heart, followed by (B) 3D reconstructions of injection sites (green) and (C) serial CT slices showing retention and stability at multiple sites over time (i–v denote injection numbers). (D–E) Gross dissection and excised tissue sections demonstrate localized nanoparticle hydrogel retention. (F) Endocardial delivery: fluoroscopic image during catheter-based injection of hydrogel into the left ventricle. (G–H) CT-based 3D reconstructions of endocardial injections in vivo and post-explant, showing hydrogel volumes (mm³) and radiopacity (HU). Reconstructions from chest cavity slightly overestimate compared to explanted tissue due to surrounding soft tissue contributions. (I) LV tissue sections confirm hydrogel localization within myocardium (dashed circles). Scale bars: 10 cm (B, G–H), 1 cm (E), 2 cm (I).

#73

Tuning the Mechanical Properties of Alginate Hydrogels for Vascular Drug Delivery

Gerardo Murgolo[1], Antonio Martino PhD [1], Sanjay Balasubramanian[1,2], Cesare Farina[1,3], Marta Aguglia[1,3], Richard C. Willson PhD[4], Maham Rahimi MD PhD[1,5,6], Carly S. Filgueira PhD[1,6]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
[2]College of Natural Science and Mathematics, University of Houston, Houston, TX, USA
[3]Department of Mechanical & Aerospace Engineering, Politecnico di Torino, Turin, Italy
[4]Department of Biochemical & Biophysical Sciences, University of Houston, Houston, TX, USA
[5]Houston Methodist Heart and Vascular Center, Houston Methodist Academic Institute, Houston, TX, USA
[6]Department of Cardiovascular Surgery, Houston Methodist Research Institute, Houston, TX, USA

Keywords: Peripheral arterial disease; Alginate–PVA hydrogel; vessel coating; Mechanical tuning; Paclitaxel release

Introduction: Percutaneous transluminal angioplasty (PTA) restores perfusion in peripheral arterial disease (PAD), but durability below the knee remains poor. Current drug-coated balloons (DCBs) are an incremental improvement over plain balloons (17.9% vs 28.6%) failure[1], yet limitations remain. They deliver a single antiproliferative drug that addresses restenosis but not acute thrombosis. Moreover, DCBs are preloaded with a fixed drug dose, limiting patient-specific adjustment, and rely on coatings that deliver drug almost instantly, with up to ~98% lost during deployment.[2] These shortcomings contribute to restenosis (20–45%) and thrombosis (3–10%) at 6–12 months.[3] To overcome these barriers, we developed a calcium alginate (CA)–polyvinyl alcohol (PVA) hydrogel for intraoperative, customizable balloon coatings.

Hypothesis: By tuning alginate precursor concentrations, PVA percent and molecular weight as well as processing temperature and time, coatings with distinct stability, adhesion, and release properties can be generated, resulting in properties suitable for vessel intima coating and drug delivery. The goal is to enable intraoperative hydrogel coatings on off-the-shelf vascular balloons, permitting therapeutic release to match patient-specific needs.

Methods: Sodium alginate (1% w/v) was crosslinked using calcium carbonate ($CaCO_3$, 15–75 mM) and glucono-δ-lactone (GDL, 30–150 mM; 1:2 molar ratio) as an initiator. PVA was incorporated at low (13–23 kDa) or high (146–186 kDa) molecular weight to tune adhesion and release. Thermal cycling (−20/55 °C) densified the hydrogel network, improving stability during sheath insertion and balloon inflation. Oscillatory rheology assessed viscoelasticity and gelation. Coatings were applied to angioplasty balloons and tested for uniformity, adhesiveness, and deployment success in pulsatile mock vessels (35 mL/min) and ex vivo porcine carotid arteries under static flow. Paclitaxel was encapsulated after either antisolvent crystallization or directly from solution, and drug release quantified by ultraviolet–visible spectroscopy (UV–Vis) and high-performance liquid chromatography (HPLC).

Results: Increasing $CaCO_3$ to GDL from 1X (15:30 mM) to 4X (60:120 mM) reduced gelation time from ~35 to ~12 min. Incorporation of 5% PVA enhanced adhesion (2.8 ± 1.1 N·s vs. 17.0 ± 1.1 N·s, $p<0.001$) without impairing rheological performance, while thermal cycling reinforced coating mechanical properties (0.29 ± 0.07 vs. 9.37 ± 3.3 %, $p<0.001$). The optimized 4X + 5% PVA coating provided continuous coverage,

tolerated sheath insertion, and transferred a ~103–294 µm layer on the vessel. Adhesiveness was confirmed in both mock vessel and ex vivo carotid arteries, with intact and non-obstructive coatings for ≥24 h under pulsatile flow. Drug release depended on formulation: low-MW PVA (13–23 kDa) enabled slower release (~53% at 5 days), while high-MW PVA (146–186 kDa) produced faster release (~79% at 5 days, Fig 1).

Conclusion: A tunable CA–PVA hydrogel enables intraoperative balloon coatings with strong adhesion, robustness, and customizable release. By varying crosslink density, PVA percent and molecular weight, and drug formulation, coatings can be tailored for different therapeutic needs. Future work will extend drug type, release duration by promoting slower-dissolving paclitaxel crystals, and leverage host–guest interactions for enhanced therapeutic retention.

Acknowledgements: Special thanks to Rebecca Swann, Daryl Schultz, and MITIE[SM] for access to supplies and for their help and guidance. This work was supported by the George and Angelina Kostas Research Center for Cardiovascular Nanomedicine.

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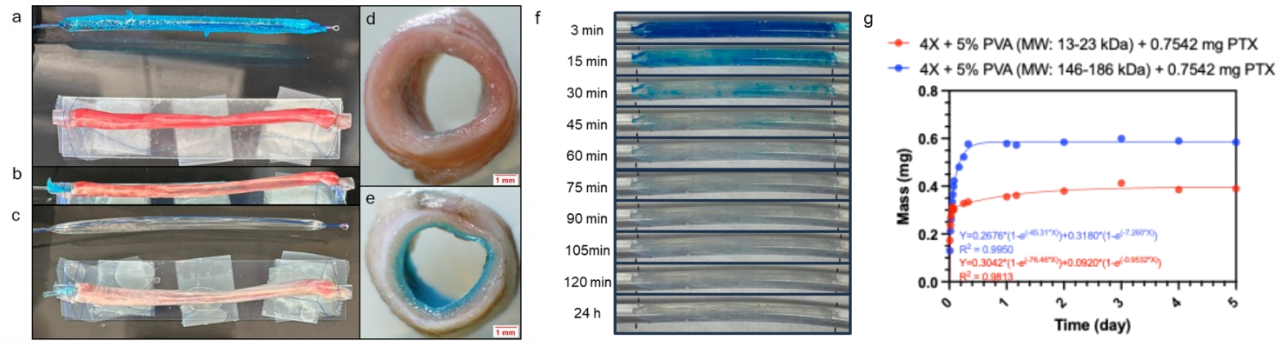

Figure 1. Characterization of the on-demand coated balloon. (a) Balloon after coating with dye for visualization. (b) Deployment of the coated balloon in an ex vivo porcine carotid artery filled with MilliQ water. (c) Balloon removed after 3 min, showing uniform coating transfer. (d) Cross-section of an uncoated artery. (e) Cross-section of the coated artery, showing a coating thickness of 294 µm. (f) Coated balloon deployed in a mock vessel under pulsatile flow for 24 h, demonstrating coating stability without obstructing flow. (g) Cumulative paclitaxel release profile from the coated balloon for two different PVA molecular weight formulations.

#74

A machine-learning-guided hydrogen-bonded organic framework for long-term, ultrasound-triggered pain therapy

Weilong He,[1†] Cathy Yang,[2†] Xi Shi,[3†] Yanxing Wang,[1†] Wenliang Wang,[1] Alexander Schafer,[2] Brinkley Artman,[1] Liwen Zhou,[2] Huiliang Wang,[1*]

[1]*Biomedical Engineering, Cockrell School of Engineering, The University of Texas at Austin, Austin, Texas 78712, United States.*

[2]*Department of Neuroscience, The University of Texas at Austin, Austin, Texas 78712, United States.*

[3]*Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, 78712, USA*

[†]These authors contributed equally to this work.

*Correspondence to: evanwang@utexas.edu

Keywords: HOF nanoparticles; ultrasound-triggered release; machine learning; local anesthetics; pain management

Introduction: Chronic pain afflicts more than 1.5 billion people worldwide and represents one of the most urgent yet unsolved medical challenges in modern medicine[1]. Existing interventions are inadequate: systemic opioids, while potent, are plagued by high risks of addiction, tolerance, and overdose, fueling a global public health crisis[1]. Conventional local anesthetic (LA) formulations, although safer, provide only short-lived relief and lack any form of spatiotemporal control, forcing patients to undergo repeated injections or systemic therapy[2]. These limitations underscore the critical unmet need for a precision analgesic platform capable of achieving high drug payload, long-term stability, and externally controllable, on-demand release. However, rational design of drug–carrier systems remains a fundamental bottleneck: empirical screening approaches are slow, resource-intensive, and rarely generalizable. Machine learning (ML) provides a transformative solution by enabling rapid, predictive modeling of drug–nanocarrier interactions, vastly accelerating discovery and expanding the accessible design space beyond what traditional trial-and-error methods can achieve.

Hypothesis: We hypothesized that hydrogen-bonded organic framework (HOF) nanoparticles, when optimized through ML-guided drug selection, could serve as ultrasound (FUS)-responsive depots for clinically relevant local anesthetics, enabling programmable, repeatable, and long-lasting pain management with unprecedented precision.

Methods: We established a ML pipeline trained on ~250 FDA-approved anesthetics and analgesics. Molecular descriptors and predicted HOF–drug interaction features were extracted and

modeled to rapidly rank candidates, compressing months of empirical testing into minutes of computation. Top-ranked candidates were validated experimentally by synthesizing TATB-based HOF nanocrystals and quantifying encapsulation efficiency[3]. Drug release profiles were measured under serum conditions and subjected to FUS stimulation to assess triggered release. Mechanistic assays included neuronal calcium imaging to confirm blockade of excitability, while *in vivo* efficacy was evaluated in rat models of sciatic nerve injury using von Frey mechanical thresholds, behavioral scoring, and motor function recovery.

Results: The ML pipeline identified lidocaine HCl and bupivacaine as optimal candidates, which achieved loading capacities up to ~30 wt%, surpassing many conventional nanocarriers. HOF–drug complexes exhibited remarkable stability, showing negligible passive leakage over 30 days. In sharp contrast, FUS stimulation induced instantaneous and repeatable release, creating programmable dosing cycles unattainable with standard formulations. Neuronal assays confirmed functional relevance: ultrasound-triggered release silenced calcium influx by blocking sodium channel–mediated excitability. *In vivo*, a single perineural injection of ML-optimized HOF–drug formulations, combined with daily ultrasound, produced robust analgesia lasting up to 7 days, far exceeding free drug controls. Treated animals demonstrated elevated withdrawal thresholds, reduced neuropathic behaviors, and improved functional recovery, validating both symptomatic and restorative benefits.

Conclusions: This work establishes the first ML-informed, ultrasound-responsive HOF nanoplatform for long-term, non-invasive pain management. ML enabled a generalizable framework for rapidly designing drug–nanocarrier systems and translating computational predictions into therapeutic function. By integrating AI-guided discovery, supramolecular chemistry, and ultrasound control, the platform delivers local anesthetics with unprecedented efficiency and precision. More broadly, it represents a paradigm shift in rational nanomedicine design, offering a versatile strategy for spatiotemporal drug release and neuromodulation.

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No Disclosures

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#77

Title: **Antibody Passivated Radiopaque Bismuth Nanoparticles for Tissue-selective Imaging**

Annalisa Crivello[1,2], Farah M. Al Hadad[1,3], Antonio Martino PhD[1], Francesco Mangano[1,2], Blake C. Fallon[1], Richard C. Willson PhD[4], Ericka P. Greene MD[5], Nilesh Mathuria MD[6], Carly S. Filgueira PhD[1,7]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA
[2]Department of Mechanical & Aerospace Engineering, Politecnico di Torino, Turin, Italy
[3]Department of Biomedical Engineering, University of Houston, Houston, TX, USA
[4]Department of Biochemical & Biophysical Sciences, University of Houston, Houston, TX, USA
[5]Department of Neurology, Houston Methodist Academic Institute, Houston, TX, USA
[6]Houston Methodist Heart and Vascular Center, Houston Methodist Academic Institute, Houston, TX, USA
[7]Department of Cardiovascular Surgery, Houston Methodist Research Institute, Houston, TX, USA

Keywords: Bismuth nanoparticles, Contrast agents, PEGylation, X-ray attenuation, Targeted imaging

Introduction: Bismuth nanoparticles (Bi Nps) are emerging as promising contrast agents for biomedical imaging. Their nanoscale size allows for cellular targeting, while bismuth's high atomic number (Z = 83) ensures strong X-ray attenuation at a lower bulk material cost compared to gold or platinum. Additionally, Bi Nps exhibit improved biocompatibility relative to iodinated contrast agents (ICA), lowering risks of hypersensitivity and nephrotoxicity.[1] Unlike ICAs, Bi Nps can be functionalized for tissue-specific targeting, such as with anti-contactin-2 antibodies for cardiac conduction system (CCS) imaging.[2]

Hypothesis: Bi Nps with optimized surface chemistry can achieve long-term colloidal stability in aqueous environments, maintain radiopacity, and enable targeted delivery for high-resolution, tissue-specific imaging.

Methods: Bi Nps were synthesized by reducing bismuth(III) nitrate pentahydrate in 1,2-propanediol using borane and glucose. Particles underwent layer-by-layer functionalization with poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), followed by PEGylation with mPEG-NH_2 via EDC chemistry.[3] Transmission electron microscopy (TEM) and dynamic light scattering (DLS) characterized particle structure and hydrodynamic diameter. ζ-potential analyses assessed surface charge and UV-vis spectroscopy measured optical absorbance and stability. PEGylation was assessed based on changes in DLS and ζ-potential as well as FT-IR spectroscopy. Using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide / sulfonated N-hydroxysuccinimide (EDC/sulfoNHS) chemistry, Bi Nps were functionalized with either Immunoglobulin G (IgG Ab)—to serve as a control group—or anti-contactin 2 (Cntn2 Ab), an antibody that targets membrane proteins in the CCS. Nano differential scanning fluorimetry (NanoDSF) was used to confirm antibody conjugation and monitor protein stability of nanoparticle-bound antibodies.

Results: The synthesized Bi Nps exhibited a hydrodynamic diameter of 105.1 ± 0.64 nm with a low polydispersity index (PDI) of 0.19 ± 0.006, as determined by DLS. TEM confirmed the preservation of a large inorganic core (~65.6 ± 9.6 nm) even after lyophilization, indicating structural integrity. ζ-potential measurements revealed a negative surface charge of −25.6 ± 0.88 mV, supporting long-term colloidal stability in aqueous media. UV-vis spectroscopy showed a consistent absorbance peak at ~350 nm over

time, confirming chemical stability. PEGylation was validated by an increase in hydrodynamic diameter (~110 to ~151 nm) and surface charge (-36 to -17 mV) as well as the presence of C-O-C and C=O peaks at 1100 cm^{-1} and ~1650 cm^{-1}, respectively. Furthermore, X-ray attenuation measurements demonstrated superior radiopacity compared to conventional iodinated contrast agents, highlighting the imaging potential of these nanoparticles. Long-term monitoring indicated that both size and surface charge remain stable after PEGylation, underscoring Np suitability for biological circulation. NanoDSF confirmed antibody presence on the Bi Nps due to evidence of protein unfolding at 70.11 ± 0.43 °C (Fig. 1).

Conclusion: The synthesized Bi Nps combine long-term aqueous stability, high radiopacity, and customizable surface chemistry. Their ability to be functionalized with antibodies enables targeted imaging applications in diagnostic and image-guided therapy. These findings support the potential of Bi Nps for in vitro and in vivo use in precision imaging.

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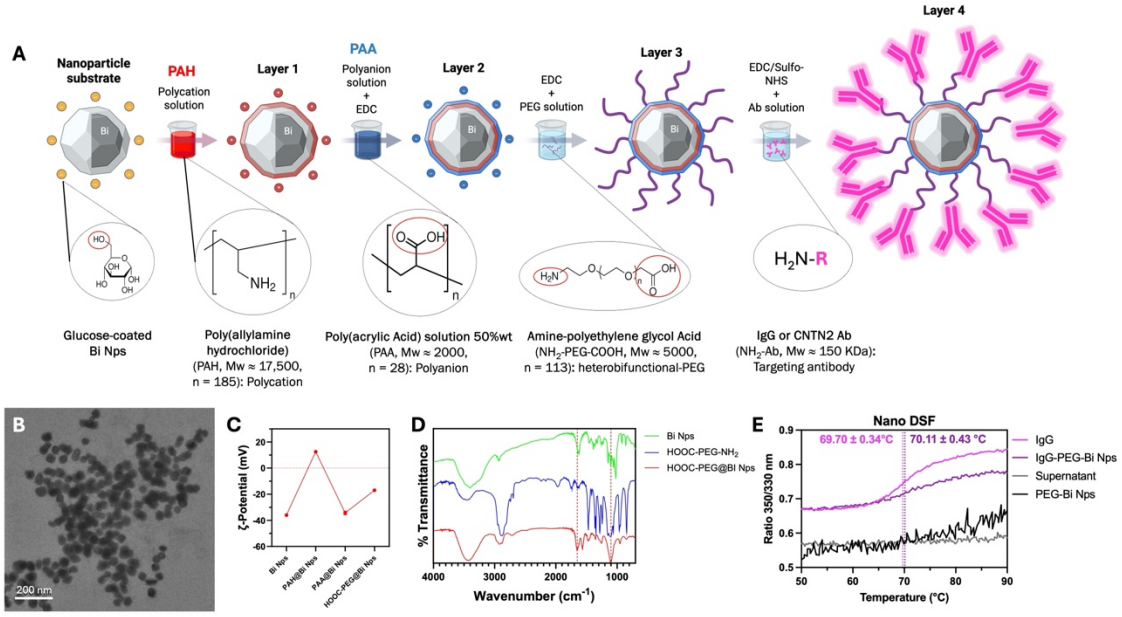


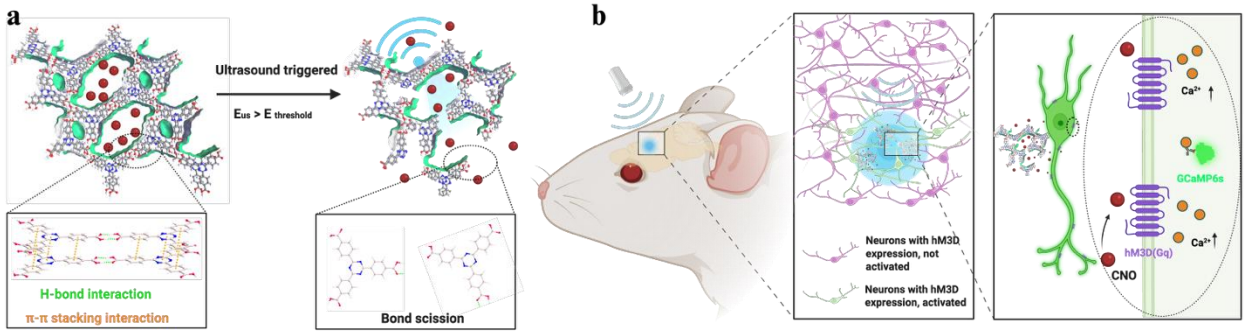
Figure 1. **Bi Nps conjugation process:** A) Schematic, B) TEM image of aqueous Bi Nps, C) zeta potential, D) FTIR spectra, and E) Nano Differential Scanning Fluorimetry (DSF) highlighting changes in protein intrinsic fluorescence as a function of temperature. ***Not to scale

#78

Title: **Non-invasive and programmable molecular manipulation for deep brain modulation using focused ultrasound**

Wenliang Wang, Yanshu Shi, Wenrui Chai, Kai Wing Kevin Tang, Banglin Chen, Huiliang Wang,

Abstract: The precise control of mechanochemical activation within deep tissues via non-invasive ultrasound holds profound implications for advancing our understanding of fundamental biomedical sciences and revolutionizing disease treatments. However, a theory-guided mechanoresponsive materials system with well-defined ultrasound activation has yet to be explored. Here we present the concept of using porous hydrogen-bonded organic frameworks (HOFs) as toolkits for focused ultrasound programmably triggered drug activation to control specific cellular events in the deep brain, through on-demand scission of the supramolecular interactions. A theoretical model is developed to visualize the mechanochemical scission and ultrasound mechanics, providing valuable guidelines for the rational design of mechanoresponsive materials at the molecular level to achieve programmable and spatiotemporal activation control. To demonstrate the practicality of this approach, we encapsulate designer drug clozapine N-oxide (CNO) into the optimal HOF small particles for FUS gated release to activate engineered G-protein-coupled receptors in the mice and rat ventral tegmental area (VTA), and hence achieved targeted neural circuits modulation even at depth 9 mm with a latency of seconds. This work demonstrates the capability of ultrasound to precisely control molecular interaction and develops ultrasound programmable HOFs to minimally invasive and spatiotemporally control cellular events, thereby facilitating the establishment of precise molecular therapeutic possibilities. We anticipate that this research could serve as a source of inspiration for precise and non-invasive molecular manipulation techniques, potentially applicable in programming molecular robots to achieve sophisticated control over cellular events in deep tissues.



Ultrasound mechanically controlled bond scissions in hydrogen-bonded organic frameworks for precise neural modulation. (a) Schematic illustration of the ultrasound mechanical stress triggered drug activation in HOFs through programmable hydrogen-bonding and π-π stacking interaction under the specific ultrasound power. (b) Ultrasound controlled designer drug (CNO) activation from HOFs for neural modulation in rat deep brain (9 mm depth).

#81

Title: Enhanced Magnetic Hyperthermia Efficiency of Iron Oxide Nanoflowers Synthesized via a Simple Co-precipitation Method.

Bosede Kolawole, Qinku Zhang, Usha Grewal, Yongfeng Zhao*

Department of Chemistry and Atmospheric Sciences, Jackson State University

Keywords: Nanoflower, Cancer therapy, Magnetic Hyperthermia, SAR

Cancer remains one of the leading causes of death worldwide, and conventional treatments such as chemotherapy and radiation often present significant side effects and limitations. As a result, there is growing interest in alternative and complementary therapies such as magnetic hyperthermia, which uses heat generated by magnetic nanoparticles to selectively kill cancer cells. In this study, iron oxide nanoflowers of varying sizes were synthesized using a simple and novel co-precipitation method. The nanoflowers were thoroughly characterized and compared with dispersed iron oxide nanoparticles of similar crystal sizes. Heating efficiency studies revealed that the nanoflowers demonstrated significantly higher specific absorption rates (SAR) than the dispersed counterparts. This enhanced performance is attributed to the unique flower-like morphology of the nanoparticles, which promotes stronger magnetic interactions and more efficient heat generation. These results highlight the potential of nanoflower-shaped magnetic nanoparticles as promising agents for magnetic hyperthermia-based cancer therapy.

#82

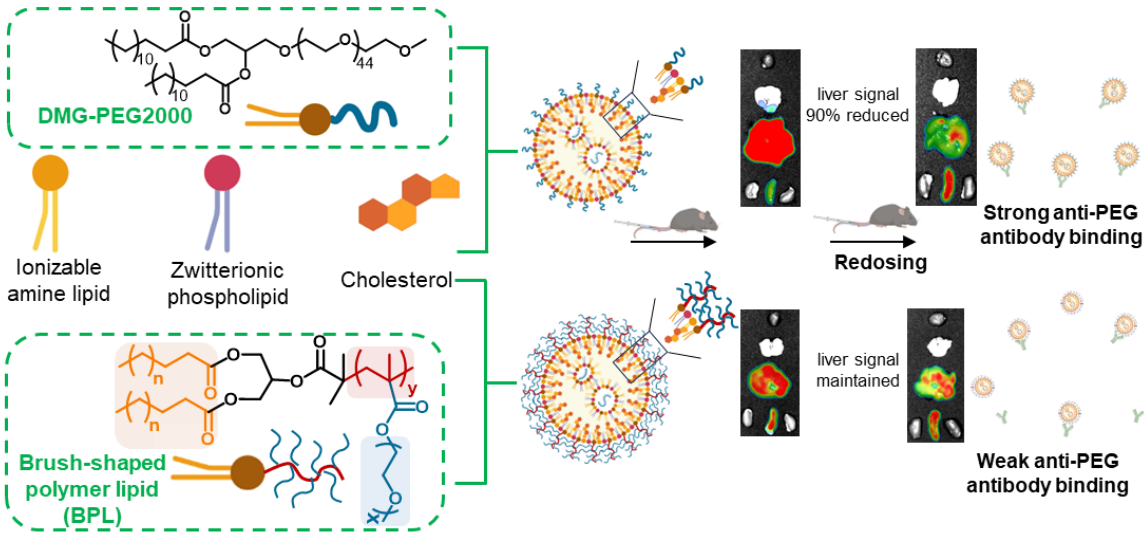
High-density brush-shaped polymer lipids reduce anti-PEG antibody binding for repeated administration of mRNA therapeutics

Yufen Xiao[1], Daniel J. Siegwart[1]

[1] Department of Biomedical Engineering, Department of Biochemistry, Simmons Comprehensive Cancer Center, Program in Genetic Drug Engineering, University of Texas Southwestern Medical Center, Dallas, TX, USA

Presenting author email address: yufen.xiao@utsouthwestern.edu

Messenger RNA lipid-nanoparticle-based therapies represent an emerging class of medicines for a variety of applications. However, anti-poly(ethylene glycol) (anti-PEG) antibodies generated by widely used PEGylated medicines and lipid nanoparticles hinder therapeutic efficacy upon repeated dosing. Here we report the chemical design, synthesis and optimization of high-density brush-shaped polymer lipids that reduce anti-PEG antibody binding to improve protein production consistency in repeated dosing. Brush-shaped polymer lipid parameters, including side chain length, degree of polymerization, anchor alkyl length and surface regimes on lipid nanoparticles modulate anti-PEG antibody binding affinity and control their blood circulation pharmacokinetics. Compared to widely used 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000, lipid nanoparticles containing brush-shaped polymer lipids generate superior therapeutic outcomes in protein replacement therapy and genome editing models, reformulating structure–activity guidelines for the design of PEG lipid substitutes. Overall, these findings contribute to the general effort in the development of lipid nanoparticles with low immunogenicity to overcome current roadblocks to nucleic acid medicines.



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#85

Cytokine mRNA-based Cancer Therapeutics

Chuang Liu[1,2], Khalid Shah[2], Wei Tao[1]

[1] Department of Neurosurgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

[2] Center for Nanomedicine and Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Keywords: mRNA, cytokine, lipid nanopartciles, cancer, immunotherapy

Daily subcutaneous injections of recombinant interleukin-10 (IL-10) demonstrated encouraging but preliminary efficacy in certain tumour types during early phase clinical trials. However, these antitumour effects were not consistently replicated in larger trials, probably due to insufficient intratumoural recombinant IL-10 accumulation, which ultimately restricted clinical benefit. Here we show that intravenous injections of IL-10 messenger RNA (mRNA) nanoparticles (*IL-10*-mRNA@NPs) induce potent immune surveillance across diverse preclinical tumour models and mitigate systemic toxicities. In particular, *IL-10*-mRNA@NPs sustain in situ IL-10 production within tumours, promoting substantial infiltration and proliferation of cytotoxic T cells, activation and maturation of dendritic cells, and an augmented expression of major histocompatibility complex class I molecules in immunosuppressive orthotopic early stage hepatocellular carcinoma tumours. Moreover, in mice with orthotopic middle-to-late-stage hepatocellular carcinoma tumours, combining *IL-10*-mRNA@NPs with immune checkpoint blockades results in 43% of mice showing complete tumour eradication and a sixfold increase in median survival compared with mice treated with immune checkpoint blockades alone. Furthermore, this combination induces long-lasting antitumour immune memory, conferring 100% protection against tumour rechallenges. The intravenous *IL-10*-mRNA@NPs strategy may haves potential to overcome the challenges associated with recombinant IL-10 in clinical trials across a broad spectrum of immunosuppressive tumours.

My first authorship/corresponding authorship publications related to this abstract:

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#86

Developing RNA-LNP therapeutics towards the CNS and Neurodegenerative Disease.

Shekinah K. Soriano, Anna K. Blakney
School of Biomedical Engineering, Michael Smith Laboratories, UBC, Vancouver.

Keywords – mRNA, saRNA, LNP, Neurogenesis, CNS

Introduction: Neurodegenerative diseases are projected to rise dramatically with the aging global population. Yet the adult brain has limited regenerative capacity, and current strategies such as stem cell transplantation face significant translational barriers, including challenges in cell sourcing, and scalability. Direct reprogramming of astrocytes into neurons (AtN) using adeno-associated viruses (AAVs) to overexpress neurogenic transcription factors (nTFs) is a promising alternative as it leverages the abundance of the surrounding cells in diseased brain regions after injury. However, AAVs have limited cargo capacity, immunogenicity, and high manufacturing costs. With the development of SARS-CoV-2 vaccines, Messenger RNA (mRNA)-LNPs have demonstrated its safety and clinically scalability and offer greater cargo capacity with transient protein expression. Self-amplifying RNA (saRNA) are next-generation mRNA that encode viral replication elements from alphaviruses, allowing greater protein expression at lower doses for up to 30 days, making this a candidate for AtN reprogramming.(1) However, translation to the central nervous system (CNS) presents major challenges such as efficient delivery across the blood-brain barrier (BBB) and avoidance of innate immune activation in the CNS microenvironment. Addressing these challenges requires integrating RNA engineering and optimizing nanoparticle delivery strategies.

Hypothesis: We hypothesize that optimal mRNA- or saRNA-LNP formulations can mediate efficient AtN reprogramming with minimal off-target effects.

Methods: To optimize RNA delivery into primary mouse astrocytes, RNA designs were quantified using flow cytometry and qRT-PCR. LNP-RNA delivery into the brain *in vivo* was optimized using MRI-guided focused ultrasound (MRgFUS). Finally, RNA-LNP mediated AtN reprogramming was evaluated using IF microscopy and qRT-PCR evaluation of neurogenic markers.

Results: We have demonstrated that RNA-LNPs can safely transfect and express protein in astrocytes and neurons *in vitro* and have optimized the backbones to have the greatest immune tolerability and viability. In parallel, we have also shown that we can deliver RNA-LNPs to the brain using MRgFUS. Finally, we have demonstrated that astrocytes transfected with saRNA encoding nTFs show increased neuronal markers and neuronal morphology.

Conclusion(s): Our results support the feasibility of RNA-LNPs as a safe and tunable platform for AtN reprogramming. Knowing this, we hope to advance the broader field of CNS therapeutics. By overcoming key challenges related to delivery, immunogenicity, and cell specificity, our work may help pave the way toward clinically viable regenerative treatments for neurodegenerative disease.

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#87

Ultrasound-Targeted Delivery of miR-27a* Loaded Microbubbles for Head and Neck Squamous Cell Carcinoma

Geetika Wadhwa [1], Nikhil S. Chari [2] , Anurag N. Paranjape [1], Xucai Chen [1], Stephen Y. Lai [2], Flordeliza S. Villanueva, MD*[1]

[1] Centre for Ultrasound Molecular Imaging and Therapeutics, Heart and Vascular Institute, University of Pittsburgh, Pittsburgh, PA, USA; [2] Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Keywords: Head and neck squamous cell carcinoma, miR-27a*, Target delivery, Ultrasound-targeted microbubble cavitation, Sonoporation.

Introduction: Head and neck squamous cell carcinoma (HNSCC) is a global health concern and ranks as the seventh most common cancer worldwide. MicroRNAs (miRs) are a promising approach for cancer therapy as these endogenous non-coding oligonucleotides bind to target mRNAs to cause post-transcriptional gene silencing. Our previous work identified miR-27a* as a regulator of multiple gene targets critical to HNSCC progression.[1] However, targeted therapeutic systemic delivery of miRs remains a significant challenge. Ultrasound-targeted microbubble cavitation (UTMC) is an image-guided platform for spatially controlled oligonucleotide delivery. Hence, we aim to develop and validate ultrasound-targeted delivery of miR-27a*-loaded microbubbles (MBs) for the treatment of HNSCC.

Hypothesis: We hypothesize that UTMC using miR-27a*-loaded MBs will deliver miR-27a* to HNSCC cells and downregulate key oncogenic signaling pathways involved in HNSCC.

Methods: Cationic lipid MBs loaded with miR-27a* or negative control (NC) miR were synthesized and evaluated based on the concentration, size, and loading, using Coulter Multisizer and gel electrophoresis.[2,3] In cultured UM-SCC-22A cells, sonoporation (a process of transient pore formation on the cell membrane due to UTMC) and viability were measured using propidium iodide and calcein-AM. To confirm cellular delivery, MBs loaded with Cy3-labeled anti-miRs were used as a fluorescent probe to trace cellular uptake. qPCR and Western blot were used to measure the effect of UTMC-mediated miR-27a* delivery on target gene expression.

Results: Cationic miR-27a*- or NC-miR- loaded MBs were successfully formulated based on charge-charge interaction. The miR-27a*-loaded MBs had an average diameter of 1.89±0.041 μ m and concentration of 1.09×10^9 MBs/mL with 84% loading efficiency. Using optimized UTMC parameters (ultrasound frequency 1 MHz, peak negative pressure 500 kPa, 10 ms pulse interval, and treatment duration 10 sec) with 10:1 MBs per cell ratio, the sonoporation rate was 12.17± 2.38% with minimal cell death (<1.5%). The Cy3-anti-miR study demonstrated that UTMC causes nucleotide permeation into cells. Furthermore, miR-27a* target genes (EGFR, AKT1, ΔNp63 α , and mTOR) were downregulated after UTMC treatment with miR-27a*-loaded MBs compared to NC-miR- loaded MBs.

Conclusion: Ultrasound-mediated targeted delivery of miR-27a* is a promising therapeutic approach for HNSCC. We successfully developed cationic MBs with high loading efficiency and optimized ultrasound parameters for efficient delivery of miR-27a*-loaded MBs into UM-SCC- 22A cells. *In vitro* analysis confirmed downmodulation of target genes after UTMC treatment with miR-27a*-loaded MBs. Future *in vivo* studies will be pivotal in determining therapeutic efficacy, dose optimization and safety for future clinical translation for HNSCC treatment.

Acknowledgement: This research work was supported by R01DE032521.

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#88

Development of tunable delayed-release microparticles and their application in single-injection vaccination for rabies post-exposure prophylaxis

Tyler P. Graf[1], Alyssa Kunkel[1], Heather Chia-Chien Hsu,[2] Katherine Chen,[1] Siamalan Krishnathas,[1] Erin Shen,[1] Kevin J. McHugh[1,2].
[1]Department of Bioengineering, Rice University, [2]Department of Chemistry, Rice University

Keywords: Single injection vaccination, Rabies, Pulsatile drug delivery, PLGA

Introduction: Rabies infections are nearly 100% fatal without intervention, resulting in approximately 60,000 deaths annually, predominantly among children in low- and middle-income countries. However, rabies is completely treatable through administration of rabies immunoglobulin and vaccinations given immediately and 3, 7, and 14 days later. The need to make four healthcare visits within two weeks exacerbates issues of limited healthcare access. There is a need to reduce the number of healthcare visits for treating rabies infections. Recent advances in microfabrication enable the generation of fully biodegradable pulsatile drug delivery microparticles, characterized by a burst release after a predetermined delay. The modularity achieved by mixing microparticles that release after different delays enables the delivery of vaccines in a single injection. Here, we develop and explore an injectable microparticle system termed Particles Uniformly Liquified and Sealed to Encapsulate Drugs (PUSLED) to enable a single injection vaccination for rabies.

Methods: Poly(lactic-co-glycolic acid) (PLGA) open-faced hollow cylindrical structures were generated through a process of 3D printing, soft lithography, and heated compression molding. These structures were filled using a piezoelectric dispensing machine with fluorescent dye conjugated to dextran, rabies vaccine, or excipients, then inverted over a heat source to seal. *In vitro* release kinetics were assessed by sampling the supernatant of particles incubated at 37°C. The release of fluorescently labeled dextran was monitored using a plate reader. *In vivo*, release kinetics were determined using an In Vivo Imaging System to monitor the release of fluorescently conjugated dextran from particles injected subcutaneously into the rear flanks of SKH1-Elite mice. Inactivated rabies vaccine stability was determined using an enzyme-linked immunosorbent assay (ELISA) that utilizes neutralizing antibodies for the capture and detection of the vaccine.

Results: Unsealed, filled, and sealed microparticles can be seen in Figure 1A. These microparticles are injectable due to their small size and can be miniaturized to fit through almost all clinically used needles (Figs. 1B and C). PULSED microparticles consist of a central depot of material fully encapsulated in a PLGA polymeric shell. This morphology enables rapid release after a tunable delay by varying the molecular weight, end group, and/or co-polymer ratio of PLGA. This is demonstrated both *in vitro* and *in vivo* (Figs. 1D-F). Rabies vaccine stability through particle fabrication and release is critical to achieving a neutralizing immune response. It was found that no significant loss of antigen occurred during dispensing or sealing of the microparticles (Figs. 1G, H). Finally, through the use of stabilizing excipients, the vaccine can be released in a pulsatile manner on days 3, 6, and 15 in an immunity-conforming state (Figs. 1I, J).

Conclusions: The properties of PLGA can be altered to tune the delay in release from PULSED microparticles. Fabrication of particles is compatible with the rabies vaccine, resulting in no loss of antigen stability. The addition of stabilizing excipients enables the release of ELISA-reactive antigen at clinically relevant time points for vaccination after rabies exposure.

#89

Poly(2-Oxazoline) Micelles as a Platform for Multi-Drug Delivery in Triple Negative Breast Cancer

Keywords: Poly(2-oxazoline), micelle, TNBC, combination therapy, physicochemical

Hallie Hutsell[a], Jacob D. Ramsey[a,b], Alexander V. Kabanov[a,c,d]

[a]*Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina, USA*
[b]*Surgical Sciences, School of Medicine, Duke University, USA*
[c]*Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina, USA*
[d]*Lineberger Comprehensive Cancer Center, University of North Carolina, USA*

Breast cancer remains the leading cause of cancer-related deaths in women younger than 50, with incidence rates steadily rising. Particularly, triple-negative breast cancer (TNBC) exhibits poor outcomes due to its lack of targetable receptors, immense tumor heterogeneity, aggressive nature, and immunosuppressive tumor microenvironment (TME). Current clinical treatment relies on a combination of systemic therapy, radiation, and surgery; however, systemic therapies are significantly limitated by poor solubility of chemotherapeutics, TME immune suppression, and the restricted efficacy of immune checkpoint blockade due to tumor heterogeneity. Consequently, the standard of care fails many patients with aggressive TNBC, underscoring the critical need for more effective systemic therapies. Our lab has developed a versatile poly(2-oxazoline) (POx) micelle delivery platform that enhances drug solubility, increases drug exposure in the tumor, and enables co-delivery of therapeutics within a single formulation.[1] Leveraging these advantages, we have previously designed formulations to successfully co-deliver chemo- and immunotherapeutics for synergistic treatment of TNBC.[2,3] Building on this strategy, we hypothesize strategic selection of chemo- and immunotherapeutics guided by synergy and physicochemical micelle properties will improve outcomes for patients with aggressive TNBC. To test this, we developed and characterized a library of multi-drug POx micelles. Our initial efforts focused on investigating drug-drug and drug-polymer interactions that govern drug retention as a result of co-loading micelles through a physiologically relevant model. We measured the key parameters of micelle formation and the drug equilibrium across micelles and serum proteins to predict relationships between co-loading and pharmacokinetic behavior *in vitro*. We aim to utilize these findings to clarify how drug physicochemical parameters and co-loading influence micelle behavior, which can be applied to formulation optimization. To further evaluate our most promising formulations, we conducted pilot studies to assess their safety and efficacy *in vivo*. Future work will involve include establishing an accurate *ex vivo* tumor-on-a-chip model, screening various ratios of these drug combinations *in vitro*, validating our screening techniques, and performing *in vivo* efficacy studies of the most promising candidates in an immunocompetent animal model. Ultimately, this work aims to develop systemic therapies for TNBC that improves the outcomes of patients with aggressive and drug-resistant tumors.

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#90

Title: Towards Precision Nanotherapeutics for Peripheral Artery Disease: Addressing Gender-Specific Disparities in Drug-Coated Balloon Delivery

Ashley Chuong[1,2], Sanjay Balasubramanian[1,3], Antonio Martino PhD[1], Richard C. Willson PhD[4], Maham Rahimi MD PhD[1,5,6], Carly S. Filgueira PhD[1,6]

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA

[2]School of Engineering Medicine, Texas A&M, Houston, TX, USA

[3]College of Natural Science and Mathematics, University of Houston, Houston, TX, USA

[4]Department of Biochemical & Biophysical Sciences, University of Houston, Houston, TX, USA

[5]Houston Methodist Heart and Vascular Center, Houston Methodist Academic Institute, Houston, TX, USA

[6]Department of Cardiovascular Surgery, Houston Methodist Research Institute, Houston, TX, USA

Keywords (Up to 5): Peripheral Artery Disease, Gender Differences, Drug Coated Balloon, Sex-specific Prevalence, Therapeutic Decision Making

Introduction: Peripheral artery disease (PAD) affects over 200 million people globally[1] and over 12 million in the U.S., with growing recognition of significant sex-specific disparities in diagnosis, treatment, and outcomes[2]. PAD is caused by arterial narrowing or occlusion, leading to ischemia, claudication, and in severe cases, limb loss.[1] Drug-coated balloons (DCBs) have improved clinical outcomes by locally delivering antiproliferative agents like paclitaxel to reduce restenosis following angioplasty. However, current DCB technologies offer fixed-dose drug delivery without accounting for individual patient factors, such as vessel size or sex-specific pharmacodynamics.

Women with PAD face delayed diagnosis due to atypical or asymptomatic presentations and experience lower primary patency rates after DCB treatment (65.4% vs. 71.8% in men at 3 years).[3] Anatomically, women often have smaller, more tortuous vessels and lower calf muscle mass, which may impair drug distribution and uptake. Hormonal differences—particularly the protective effects of estrogen prior to menopause—also influence vascular remodeling and healing. Despite these factors, women remain underrepresented in PAD clinical trials, limiting the ability to develop evidence-based, sex-specific treatment strategies.

Hypothesis: We propose that integrating sex-specific anatomical and physiological insights into drug delivery system design—such as adjustable-dosage, nanoengineered catheters—can improve therapeutic outcomes for both women and men with PAD.

Methods: A systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to evaluate sex-based differences in PAD presentation, diagnosis, and DCB treatment outcomes. Databases including PubMed and Google Scholar were searched for peer-reviewed studies examining disparities in vascular access, drug response, and procedural complications. The ankle-brachial index (ABI) was analyzed as a diagnostic tool to estimate PAD prevalence by sex (Figure 1).

Results: Studies reveal higher PAD prevalence in women over 40, with disease progression linked to hormonal changes post-menopause. Women are less likely to be prescribed standard therapies (e.g., statins, antiplatelets) and are more prone to complications from percutaneous interventions—including access site hematomas, dissections, and higher mortality rates. These disparities are compounded by fixed-dose DCBs, which may result in overdosing in smaller vessels or underdosing in calcified lesions. The current "one-size-fits-all" model of drug delivery fails to address the unique vascular biology and clinical needs of women with PAD.

Conclusion(s): There is a critical need for next-generation, personalized drug delivery systems leveraging nanotechnology and adaptable catheter platforms to optimize therapy for diverse PAD populations. Sex-specific anatomical and physiological factors should inform the design of future DCBs and drug delivery strategies to ensure safe, effective, and equitable vascular interventions.

Acknowledgements: We would like to acknowledge the Brown, Smith & Raymond EnMed Capstone Innovator Award for funding.

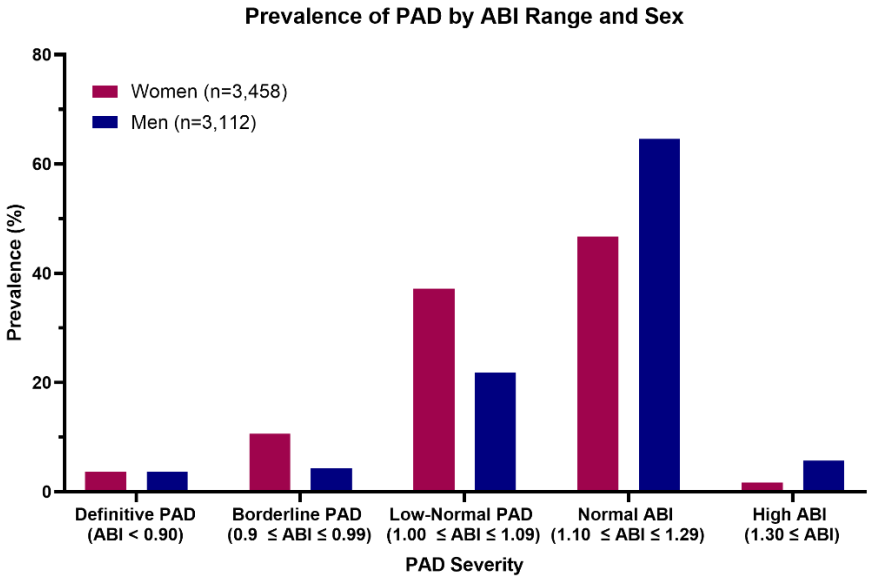

Figure 1: Prevalence of peripheral arterial disease (PAD) categorized by ankle-brachial index (ABI), a non-invasive test used to assess blood flow in the legs, for women (red) and men (blue). Data indicates a greater incidence of PAD in males with normal and high ABI while for females PAD incidence is greater when ABI is low.

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#91

Title: **Insertion of Penicillin Binding Protein (PBP4) in Nanodiscs and Kinetic Assessments with LiaX for Insights into Antibiotic Resistance**

Madison M. Bowden[1], Kara S. Hood[2,3,#], Ana P. Streling[2,3], William R. Miller[2,3,4], Cesar A. Arias[2,3,4], Carly S. Filgueira[1,5,6,#]

[1]Department of Nanomedicine, Houston Methodist Academic Institute, Houston, TX, USA
[2]Department of Infectious Disease, Houston Methodist Hospital, Houston, TX, USA
[3]Department of Infectious Disease, Houston Methodist Academic Institute, Houston, TX, USA
[4]Department of Medicine, Weill Cornell Medicine, New York, NY, USA
[5]Department of Cardiovascular Surgery, Houston Methodist Academic Institute, Houston, TX, USA
[6]Department of Nanomedicine in Cardiothoracic Surgery, Weill Cornell Medicine, New York, NY, USA

[#]Corresponding Author(s): Carly S. Filgueira, Houston Methodist Research Institute, 6670 Bertner Ave, Houston, TX 77030 Email(s): csfilgueira@houstonmethodist.org and kshood@houstonmethodist.org

Keywords: Antibiotic resistance, nanodiscs, binding kinetics, penicillin-binding proteins, enterococcus

Introduction: Enterococci are a common cause of infections in healthcare settings. Vancomycin-resistant enterococci (VRE) infections are treated with the last-resort antimicrobial lipopeptide daptomycin (DAP) but resistance to DAP (DAP-R) has emerged. The onset of DAP-R coincides with increased susceptibility to β-lactams, to which enterococci are usually resistant. This trade-off between resistance is known as the "seesaw" effect. In *Enterococcus faecalis*, the protein LiaX acts as the fulcrum of the seesaw effect through direct interaction with penicillin binding protein 4 (PBP4). Here, we investigate the binding dynamics between truncated and full-length LiaX and PBP4 to understand the mechanistic basis for the seesaw effect.

Hypothesis: The proposed seesaw mechanism links LiaX binding with PBP4's affinity for β-lactams. However, the specific domains of LiaX (N-terminal vs C-terminal) that bind PBP4 and how this regulates antibiotic affinity are not known. We *hypothesize* that direct interaction of full-length LiaX (FL-LiaX) with PBP4 affects its affinity for β-lactam antibiotics.

Methods: Full-length PBP4 was purified through affinity chromatography and inserted into 10nm lipid nanodiscs comprised of his tagged MSP1D1 belt protein and 16:0-18:1 phosphatidylglycerol. PBP4 and nanodiscs were evaluated through dynamic light scattering (DLS) and differential scanning fluorimetry (DSF). PBP4 folding within the nanodisc was confirmed through DSF and binding to a fluorescent β-lactam derivative bocillin-FL (BOC-FL). PBP4 interaction with purified, truncated (Nt-LiaX) or FL-LiaX was characterized for binding affinity and kinetics by surface plasmon resonance (SPR).

Results: We show that the 75 kDa solubilized PBP4 protein has a melting temperature of 59.03 ± 0.06°C, which increases to 60.97± 0.02°C when incorporated into a nanodisc, suggesting increased stability of the protein when inserted into a lipid bilayer. Occupied PBP4 nanodiscs have an average radius of 7.66 ± 0.21 nm, larger than that of empty discs (4.20 ± 0.09 nm). Both solubilized PBP4 and PBP4 in nanodiscs were able to bind to BOC-FL, indicating proper folding of the β-lactam binding pocket. SPR analysis of PBP4 in nanodiscs with FL-LiaX showed a K_{on} of 5.9E3 $M^{-1}s^{-1}$, a measured binding affinity (Kd) of 760 nM, and a K_{off} of 0.0021 s^{-1}. SPR with Nt-LiaX showed a K_{on} of 1.4E6 $M^{-1}s^{-1}$, a Kd of 3.1 μM, and a K_{off} of 2.7 s^{-1}. Association

time of FL-LiaX with PBP4 in nanodiscs was calculated to be 489 s, whereas with Nt LiaX the association time was 0.37 s.

Conclusion: Understanding the molecular mechanisms directing protein complexation and antibiotic binding is critical for effective antimicrobial stewardship and enhancing combinatorial therapy to prevent resistance. Our hope is for this research to improve clinical outcomes, reduce hospital length of stay, and decrease healthcare costs.

Acknowledgements: This project is supported by Nanomedicine and Infectious Diseases Pilot Funding awarded to KH and CSF and well as by the Department of Defense (DoD) under award number HT9425-25-1-0059. We acknowledge Dr. Kelly Churion and the GCC IDDD core at TMC3, assistance for the work came from the core and that the core was supported by Cancer Prevention and Research Institute of Texas grant RP250505. We would like to thank Ayesha Khan for preliminary data gathering on the interaction between Pbp4 and LiaX.

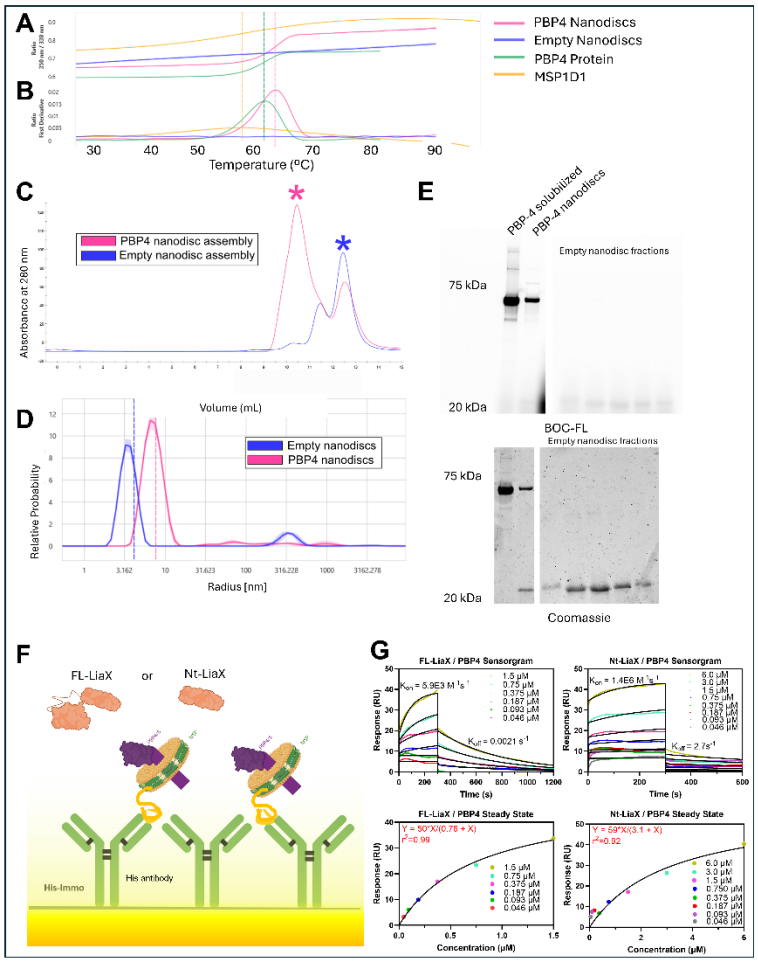

Figure 1. **Insertion of PBP4 into nanodiscs and interaction with FL-LiaX and Nt-LiaX.**

#92

Title: Fiducial Marker and Delivery Platform for Guiding and Monitoring Cardiac Nanomedicine Therapies

Authors: Deeksha Sarda*1, Clayton Wiggins[1], Rohan Gupta[1], Flordeliz Bowles[1], Maan Malahfji, MD[2], John S. Wilson, MD PhD[1], Nilesh Mathuria, MD[2], Carly S. Filgueira, PhD[2]

[1]School of Engineering Medicine, Texas A&M University, Houston, TX 77030

[2]Houston Methodist Hospital, Houston, TX 77030

Keywords: Fiducial Markers, Nanomedicine, Drug Delivery, Cardiac Therapy

Introduction: Myocardial infarction continues to be a significant driver of heart disease, both in the acute setting and through chronic changes to the myocardium through fibrotic remodeling (1). Targeted nanomedicines offer the potential to reduce chronic disease with anti-fibrotic or regenerative medicines but still require the ability to track and guide their delivery and monitor their retention within the changing myocardium. Current techniques lack the capacity to precisely co-register targeted tissue and potential drug delivery systems across various clinically relevant modalities, such as magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound (US) (2). Further, no existing device enables temporary marker localization on the endocardial surface in a way that is both stable and minimally invasive. Current strategies rely on external markers, surgical or sutured placement, or myocardial penetration, methods which are often imprecise, invasive, or susceptible to dislodgement by cardiac and respiratory motion (3). This work evaluates optimal fiducial marker (FM) materials for multimodal imaging and explores the development of delivery systems to improve co-registration.

Hypothesis: We hypothesize that a fiducial marker, visible across multiple imaging modalities, can provide stable, artifact-free reference points to accurately localize and guide the delivery of therapeutic nanomedicines to cardiac scar tissue, and that a catheter-based system can be developed to deliver, adhere, and retrieve the fiducial in a stable and minimally invasive manner to enable accurate and temporary localization during imaging procedures without collateral vessel or tissue damage.

Methods: Six fiducial markers of varying materials and geometries were evaluated in a tissue-mimicking phantom: a commercial CT-SPOT®, an MR-SPOT®, a helical nitinol coil, a nitinol wire, a gold marker, and a fish oil within a plastic capsule. Markers were imaged using a suite of clinical scanners, including high-field (3T) MRI and computed tomography (CT). MRI sequences included T1 MPRAGE, T1 Fat-Saturated, T2 FLAIR, T2 SE, T2 HASTE, T2 TRUFI-SSP, T2 STIR, T2 Black-Blood, T2 TSE-Fat-Saturated, and Proton Density sequences. A design exploration framework will be employed to systematically develop and test catheter-based delivery systems for stable fiducial marker placement and retrieval.

Results: Nitinol-based markers, in both coil and wire geometries, demonstrated superior performance, providing the best visibility across all platforms (3T AND 1.5T MRI). In contrast, the gold marker produced significant artifacts on CT, which would obscure underlying tissue detail. Fish oil demonstrated strong visibility on MRI but was poorly detected on CT. MR-SPOT® and

CT-SPOT® were well-defined within their intended imaging modality and moderately defined on the other.

Conclusions: Nitinol is an optimal material for developing cardiac fiducial markers intended for multimodal applications. When integrated with minimally invasive delivery mechanisms, these markers can establish a powerful platform technology for tracking nanotherapeutics [3]. This system directly addresses the critical need for precise guidance and non-invasive, longitudinal monitoring of advanced cardiac therapies, potentially accelerating the development and clinical adoption of next-generation nanomedicines for treating heart disease.

Acknowledgements: This research was supported by the Brown, Smith & Raymond EnMed Capstone Innovator Award.

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#93

Title: Enhanced anticancer efficacy of lipid nanoparticle-delivered anti-PD1 antibody is dependent on myeloid immune cells.

Authors: Md Rakibul Islam[A], Jalpa Patel[A], Patricia Back[A], Shadan Modaresahmadi[A], Chidiebere Ezeh[A], Ninh M. La-Beck[A,B]

[A]Department of Immunotherapeutics and Biotechnology, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center, Abilene, Texas 79601, USA

[B]Department of Pharmacy Practice, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center, Abilene, Texas 79601, USA

Keywords: PD-1, nanoparticle, immunotherapy, tumor macrophage

Introduction: Systemic anti-PD1 therapy is often limited by off-target effects that can trigger severe immune-related toxicities and limit clinical responses[1]. Recent evidence indicates that, in addition to T-cells, PD-1 is expressed on myeloid cell subsets, including circulating monocytes, dendritic cells, myeloid progenitors, and tumor-associated macrophages (TAM)[2]. Lipid nanoparticle (LNP) carriers offer a strategy to sustain anti-PD1 antibody exposure within the tumor microenvironment to improve therapeutic efficacy. Their preferential uptake by myeloid cells can be exploited to enhance drug delivery to TAMs and stimulate anti-tumoral immune response[3]. In this study, we leverage LNP interactions with myeloid cells to overcome the low response rates while avoiding off-target effects by conjugating anti-PD1 to liposomes. Our goal is to develop an LNP formulation with high anti-PD1 conjugation efficiency, long-circulating half-life, and increased efficacy against melanoma over conventional anti-PD1 antibody.

Hypothesis: We hypothesize that liposomal anti-PD1 (LAP) will enhance the anti-tumoral effects of PD-1 blockade by targeting PD-1 on myeloid immune cells.

Methods: C57BL/6 mice implanted with B16-F10 tumor cells were injected intravenously with 10 mg/kg of either free anti-PD1 antibody, LNP-isotype, LAP, or equivalent volume of vehicle to assess LAP pharmacokinetics (PK). Therapeutic efficacy was evaluated in the same model with treatments or controls administered every five days until study endpoint. Mice were monitored for morbidity and tumor growth and tissues were collected at endpoint for PK analyses, cytokine analyses, immunophenotyping by multiparameter flow cytometry and histopathology. The role of myeloid immune cells was tested in CD11b-depleted and mock-depleted mice.

Results: Physicochemical analyses of LAP confirmed anti-PD1 antibody conjugation with an average particle size of 89 nm and a yield of up to 72% of antibody after terminal sterilization. The plasma concentration-time curve of LAP exhibits a bi-phasic elimination of anti-PD1 antibody, where the terminal phase represents 71% of the curve with a half-life of 33 days, compared to a single-phase and 31 hours half-life for free anti-PD1 antibody. The initial phase of the curve coincided with a systemic increase in inflammatory cytokines including GM-CSF, G-CSF, and IL-21. LAP significantly increased overall survival and significantly reduced melanoma growth, achieving complete tumor rejection in 67% of mice compared to 17% for the free antibody. LAP polarized TAMs towards an anti-tumoral phenotype and significantly reduced regulatory T-cells while increasing the proportion of cytotoxic to regulatory T-cell population. Depletion of $CD11b^{+}$ myeloid cells abrogated the enhanced efficacy of LAP without a major change in LAP PK. LAP did not increase systemic or organ-specific autoimmune toxicities compared to conventional PD-1 antibody blockade.

Conclusion: LAP is a rapidly translatable formulation with improved anti-tumor efficacy compared to the conventional anti-PD1 antibody without exacerbating autoimmune toxicities in a murine melanoma model. Mechanistic studies support that the LAP enhanced efficacy is due to targeting of myeloid immune cells. LAP has broad applications as a platform for combination immunotherapy and chemoimmunotherapy in the treatment of cancer.

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#94

Title: Fenofibrate Microemulsion Eyedrop for Treating Mustard Gas-Induced Corneal Injuries

Ehsan Kaffash, PharmD[1], Rudra Pangeni, PhD [1], Wentao Liang, PhD [2], Sagun Poudel, PhD [1], Jian-xing Ma, MD, PhD [2] and Qingguo Xu PhD [1].

[1] Department of Pharmaceutics, Virginia Commonwealth University, Richmond, Virginia 23298, United States

[2] Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Keywords: PPARα agonist; Vesicants; Ocular injury; Eyedrop; Ocular drug delivery

Introduction:

Fenofibrate, a peroxisome proliferator-activated receptor α (PPARα) agonist, has been used clinically for the treatment of dyslipidemia for over 30 years [1]. The role of PPARα in mitigating oxidation, inflammation and angiogenesis has previously been established. Furthermore, PPARα is highly expressed in the cornea [2]. Nitrogen mustard (NM) is a potent vesicating chemical warfare agent that can cause severe corneal injuries [3]. This study aims to formulate and evaluate fenofibrate microemulsion (Feno ME) eyedrop for treating NM-induced corneal injuries.

Methods:

0.5% Feno ME eyedrop formulation was prepared using the high-energy emulsification method. The formulation was then characterized for droplet size, polydispersity index (PDI), zeta potential, osmolarity, rheological behavior, and drug loading. The safety profile of the eyedrops was evaluated by investigating the changes in blink rate, intraocular pressure (IOP) and tear secretions following Feno-ME eyedrop administration (3x per day). NM-induced corneal injury was established on SD rats through the topical application of 1% NM solution for 2 mins. Then SD Rats with NM-induced corneal injury were treated with Feno ME, Placebo ME and saline eyedrops (3× per day, n=14 eyes each group). Routine clinical observations and evaluations for corneal opacity, corneal ulceration, and corneal neovascularization were performed on day 14 post injury. Histological analysis was conducted to assess corneal integrity following treatment with Feno ME.

Results:

The prepared Feno ME eyedrop had a droplet size of 20.4 ± 0.1 d.nm, PDI of 0.07 ± 0.01, a near-neutral zeta potential (-0.7 ± 0.4 mV), an osmolarity of 457 mOsmol/kg, and a drug loading of above 95.6 %. Rheological analysis revealed that Feno ME eyedrops maintain high viscosity at corneal temperature (35 °C), supporting enhanced retention on the ocular surface. Their shear-thinning behavior, with high viscosity at low shear rates and low viscosity at high shear rates, suggests prolonged residence time without causing blinking discomfort. Safety evaluations showed no signs of toxicity or irritation to the eyes, nor changes in blink rate, tear secretion, or IOP after 7 days eyedrop administration. Efficacy studies revealed that Feno ME eyedrop significantly inhibited corneal ulceration (1.2-fold, $P<0.05$), neovascularization (1.7-fold, $P<0.001$), and opacity (1.8-fold, $P<0.001$) over a duration of two weeks in rats with NM-exposed corneas compared to saline treated group. On the other hand, the group treated with placebo ME eyedrop significantly developed severe corneal ulceration (1.2-fold, $P<0.01$), neovascularization (1.4-fold, $P<0.001$), and opacity (1.5-fold, $P<0.01$) compared to Feno ME eyedrop treated group. In addition, histology evaluations revealed that Feno ME eyedrop significantly inhibits epithelial degradation ($P<0.001$) and decrease total corneal thickness (18-20%) compared to saline ($P<0.01$)

and placebo ME eyedrop ($P<0.05$) in NM-injured corneas, in which the corneal epithelia were degraded with extensive inflammation in the corneal stroma.

Conclusions:

Feno ME eyedrops could serve as a promising treatment option with potential for easy translational application in the treatment of corneal injuries induced by vesicants.

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#95

Nanoparticle Contrast-Enhanced CT for Early Detection and Staging of Aortopathy

Laxman Devkota,[1,2] Renuka Menon,[1,2] Prajwal Bhandari,[1,2] Chen Zhang,[3] Deborah Vela,[4] Zhen Zhou,[7] Prasad Admane,[1,2] Rohan Bhavane,[2] Andrew Badachhape,[1,2] Zbigniew Starosolski,[1,2] Scott A. LeMaire,[3,5,6] Ying H. Shen,[3,5,6] Dianna Milewicz,[7] Ketan B. Ghaghada[1,2,6]

Affiliations
[1]*Department of Radiology, Baylor College of Medicine, Houston, TX.*
[2]*Edward B. Singleton Department of Radiology, Texas Children's Hospital, Houston, TX.*
[3]*Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX.*
[4]*Department of Cardiovascular Pathology Research, Texas Heart Institute, Houston, TX.*
[5]*Texas Heart Institute, Houston, TX.*
[6]*Cardiovascular Research Institute, Baylor College of Medicine Houston, TX.*
[7]*UT Health Science Center, Houston, TX.*

Introduction: Aortopathy refers to a spectrum of disorders, including aneurysm, dissection, and rupture, that affect various segments of the aorta. Over half of aortic dissections occur in individuals with aortic diameters below the surgical threshold, and some even arise in patients with normal aortic size. This underscores the need for non-invasive imaging techniques capable of detecting early-stage aortopathy to improve patient management and predict disease progression more accurately. In this preclinical study, we evaluated the utility of nanoparticle contrast-enhanced CT (nCECT) for early detection and staging of aortopathy.

Methods: *In vivo* studies were performed in mouse models of sporadic aortic aneurysm and dissection (AAD), induced either by subcutaneous infusion of angiotensin II (Ang II) or oral administration of β-aminopropionitrile (BAPN). A liposomal-iodine (Lip-I) nanoparticle contrast agent was used for nCECT imaging. Mice received intravenous Lip-I followed by delayed CT (CTD) imaging five days post-injection. Immediately afterward, a second Lip-I dose was administered, and CT angiography (CTA) was performed to anatomically localize CTD findings. At study completion, mice were euthanized, and their aortas were harvested for gross examination, ex vivo nCECT, and histopathological analysis.

Results: CTD revealed a higher incidence of aortopathy compared to gross examination in both mouse models. Intramural CT signals—indicative of Lip-I accumulation within the aortic wall due to compromised barrier integrity—were consistently observed. These findings correlated with aortic degeneration on gross examination and intimal-medial tears on histology (Figure 1). Importantly, CTD detected intramural signals in aortic segments that appeared normal on both CTA and gross examination. In the Ang II model, CTD improved detection rates by 20%. In the BAPN model, intramural signal intensity on CTD correlated with disease stage: mild during early degeneration, moderate-to-high during active degeneration, mild-to-moderate during active repair, and negligible-to-mild in the healed stage.

Conclusion: nCECT enabled early detection of aortopathy prior to vessel enlargement and overt anatomical changes. Furthermore, the presence and intensity of intramural CT signal correlated with the disease stage, suggesting nCECT as a promising tool for staging and monitoring aortopathy progression.

#96

Stereoselective Interactions of Chiral Polyurea Nanocapsules with Albumins

Amani Zoabi[1], Adan Sultan[1], Malak Abo Alhija[1], Sergei Remennik[2], Anna Radko[2], Katy Margulis[1]*

[1] The Institute for Drug Research, the School of Pharmacy, the Faculty of Medicine, The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 9112192, Israel

[2] The Unit for Nanoscopic Characterization, The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Keywords: Chirality, Polyurea nanocapsules, Albumin adsorption, Stereoselective interactions, Cellular internalization.

Introduction

Chirality in nanostructures is increasingly recognized for its role in mediating specific biological interactions, yet intrinsically chiral nanocarriers remain scarcely explored in drug delivery. This study examines chiral polyurea nanocapsules synthesized from d- and l-lysine, revealing how their configuration and morphology influence stereoselective interactions with albumins—critical plasma proteins governing pharmacokinetics and cellular uptake.Chirality plays a key role in biomolecular interactions, but its use in the design of intrinsically chiral nanocarriers for drug delivery is just emerging. This study evaluates polyurea nanocapsules synthesized with d- or l-lysine, focusing on how chiral configuration affects albumin binding and cellular internalization—properties vital to optimizing pharmacokinetics.

Hypothesis

Chiral polyurea nanocapsules interact differentially with albumins based on their enantiomeric configuration, primarily due to inherent differences in capsule surface morphology, leading to distinctive protein adsorption and cellular uptake profiles.

Methods

Chiral nanocapsules were synthesized through interfacial polymerization between toluene 2,4-diisocyanate and either d- or l-lysine, in a volatile oil-in-water emulsion, followed by solvent evaporation. Capsule size was refined to match in vivo application needs. Protein interactions were studied via dynamic light scattering (size changes), fluorescence quenching, and zeta potential analysis. Transmission electron microscopy (TEM), atomic force microscopy (AFM), and energy-dispersive X-ray spectroscopy (EDS) characterized capsule morphology. Biocompatibility, stability, and cellular uptake were quantified using standard in vitro assays.

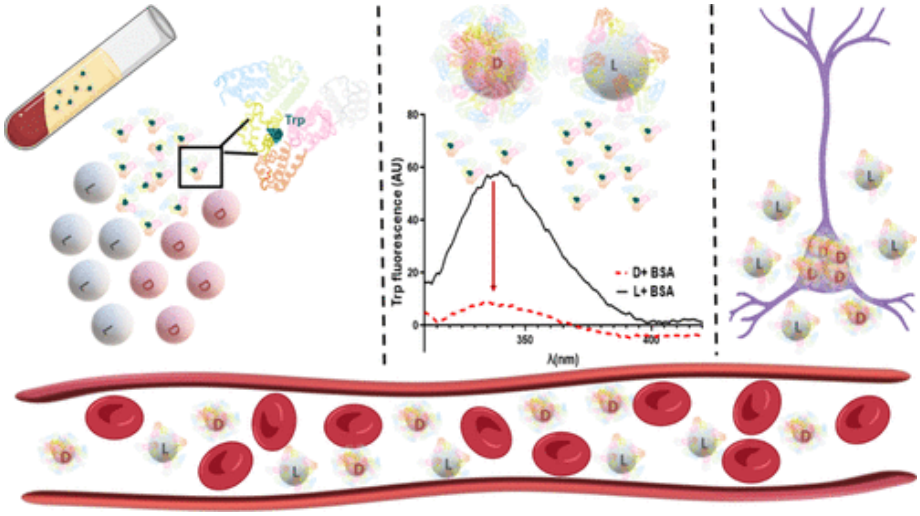
Results

D-nanocapsules exhibited 2.1–2.6 times greater albumin adsorption compared to l-nanocapsules, attributable to their more concave, rough-surfaced morphology. Modulating the ratio of d- and l-lysine during synthesis enabled fine-tuning of albumin

adsorption. Both enantiomeric forms displayed excellent stability and biocompatibility in vitro. Importantly, d-nanocapsules demonstrated a 2.5-fold increase in cellular internalization versus l-nanocapsules, suggesting chirality-driven differences in protein corona formation mediate cellular uptake.

Conclusion

Our work reveals that nanocapsule chirality and morphology profoundly affect protein adsorption and cellular uptake, providing new strategies for controlled pharmacokinetics in nanomedicine. Adjustable chiral composition enables fine-tuning of nanocarrier–protein interactions, offering valuable design options for next-generation drug delivery systems.



Acknowledgements

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#97

Poly(2-oxazoline) Micelles: A Transformative Technology for Breast Cancer Therapeutics Enabling High-Bioavailability Oral Paclitaxel

Jacob D. Ramsey [1, 2, 3], Hallie Hutsell [2], Ayse Baysal [3], Bruce Frank [3], Alexander V. Kabanov [2]

[1] Surgical Sciences, Department of Surgery, Duke University School of Medicine; [2] Center for Nanotechnology in Drug Delivery, Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, UNC Chapel Hill; [3] DelAQUA Pharmaceuticals, Chapel Hill, NC

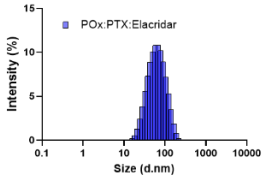
Keywords: Oral Delivery, Paclitaxel, Pharmacokinetics, Modeling, Breast Cancer

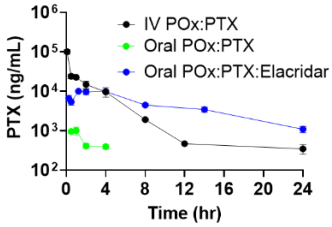
Introduction: Paclitaxel is a first and second line therapy for several oncology indications. Current administration requires a hospital visit and long infusion times which is a barrier for patients, costly, and requires extensive input from healthcare professionals. An alternative oral dosage form would be preferable and improve patient compliance while allowing patients to take their chemotherapy in the comfort of their own home. However, Paclitaxel is a Pgp substrate, meaning it is actively pumped back into the intestinal lumen as it tries to cross the intestinal lining. In this work, we co-load paclitaxel with a Pgp inhibitor in our Poly(2-oxazoline) micelles which positions the drug closer to the intestinal lining and at higher concentrations to help drive diffusion into the blood stream.

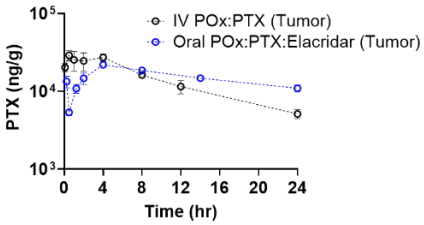
Hypothesis: We hypothesize that loading paclitaxel into our poly(2-oxazoline) micelles will enable delivery through oral administration by co-encapsulation with Pgp inhibitors which will allow paclitaxel to navigate the intestinal environment and reach circulation.

Methods: Paclitaxel was co-loaded with Pgp Inhibitors to enhance the oral delivery of paclitaxel. Mice were dosed by oral gavage and tritium labeled paclitaxel concentrations were measured in the plasma after administration. Pharmacokinetic models were developed with tumor effector compartments to estimate tumor exposure from alternative daily dosing regimens which could improve patient compliance.

Results: Co-loading Paclitaxel and Elacridar (Pgp inhibitor) into the same poly(2-oxazoline) micelles yielded narrowly disperse polymeric micelle nanoparticles which were administered to mice via oral gavage. A study in tumor bearing mice demonstrated an unprecedented near-100% oral bioavailability of paclitaxel with equivalent tumor exposure. PK models were built to simulate alternative dosing regimens that could mitigate toxicities through daily dosing while maintaining efficacy.







Formulation	Cmax (ng/mL)	AUC-last (ng*hr/mL)	F (%)
IV POx:PTX	102000	114614	-
Oral POx:PTX	1015	3790	3.31%
Oral POx:PTX:Elacridar	10008	110493	96.40%

Formulation	Cmax (ng/mL)	AUC-last (ng*hr/mL)	AUC-Last Tumor/Plasma Ratio -
IV POx:PTX	28985.4	337003	2.94
Oral POx:PTX:Elacridar	21899.9	365944	3.31

Conclusions: We have developed a drug delivery system enabling unprecedented delivery of paclitaxel by oral delivery nearing 100% oral bioavailability. This data suggests a clinically viable formulation which could reduce healthcare costs and improve patient experience.

References: *Unpublished data. Manuscript in preparation.*

#98

Simultaneous Profiling Proteinopathy and Neuroinflammation in the A53T mouse Model via a Precision Imaging Nanoparticle Probe: Dual Molecular MRI of Parkinson's Disease Pathology

Xianwei Sun[1], Andrew Badachhape,[1] Prajwal Bhandari,[1] Jeannie Chin,[2] Ananth Annapragada,[1,3] Eric Tanifum[1,3*].

[1]Department of Radiology, Baylor College of Medicine, Houston, TX 77030, USA

[2]Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA

[3]Department of Radiology, Texas Children's Hospital, Houston, TX 77030, USA

*Corresponding author: Eric Tanifum, tanifum@bcm.edu

Background: The pathogenesis of Parkinson's disease (PD) is characterized by progressive deposition of alpha-synuclein (α-syn) aggregates in dopaminergic neurons and neuroinflammation. Noninvasive *in vivo* imaging of α-syn aggregate accumulation and neuroinflammation can elicit the underlying mechanisms involved in disease progression and facilitate the development of effective treatment as well as disease diagnosis and prognosis. We present a new approach to simultaneously profile alpha-synuclein (α-syn) aggregation and reactive microgliosis *in vivo*, by precision targeting of oligomeric α-syn in cerebrospinal fluid with liposomes bearing a magnetic resonance imaging (MRI), contrast payload.

Method: Oligomeric α-syn targeting liposome (**DPDx**) formulation with Gd(III)DSPE-DOTA as the MRI contrast payload, Rhodamine lipid as a fluorescence reporter, **DSPE-PEG3400_XW-01-11** as targeting moiety which includes the highly specific α-syn aggregate binging ligand **XW-01-11**[1] was prepared using standard hydration/extrusion protocols. In vitro nanoparticle cell uptake studies were conducted with HMC-3 cells as a microglia cell line and differentiated SHSY5 cells as a neuron cell line. In vivo mouse experiments were conducted in the A53T α-synuclein transgenic line M83 mouse model (n=6). The probe was administered via the tail vein at a dose of 0.1 mmol Gd/kg body weight. Controls included wildtype mice administered the targeted agent (n=6) and transgenic controls (n=6) injected with a nontargeted variant of the agent (**N**). Mice were pre-scanned to establish a baseline followed by injection of the agent. Post-contrast scans were obtained at 4 days post-injection with the parameters as the pre-scans: *SE parameters*: TR = 600 ms, TE = 11.5 ms, slice thickness = 1.2 mm, matrix = 192 × 192, FOV = 30 mm, slices = 16, NEX = 4; *FSE-IR parameters*: TR = 13500 ms, TE = 80 ms, TI = 2000 ms, slice thickness = 2.4 mm, matrix = 192 × 192, FOV = 30 mm, slices = 6, NEX = 6. Following the final scans, Animals were euthanized, and brains removed for ex vivo immunohistochemical analysis.

Result: *In vitro* experiments showed that **DPDx** formed agglomerates upon exposure to synthetic α-syn fibrils, and microglia cells and neuronal cell lines showed accelerated uptake of the nanoparticles compared to controls. *In vivo* MRI of transgenic mice at 4 days post contrast administration showed higher MRI signal enhancement in the olfactory bulb, cortex, and brain

stem of transgenic mice treated with **DPDx** (**TgT**) compared to wild types (**WtT**) and transgenic mice treated with **N** (**TgN**). *Ex-vivo* immunohistochemical analysis showed the nanoparticles in the cytosolic compartment of IBA1 reactive cells (microglia) in the A53T α-synuclein transgenic line M83 mouse model.

Conclusion: The results demonstrate that liposomes can cross the BBB into the CSF and label enables noninvasive separation of disease mice from controls using MRI. This novel approach has the potential to significantly impact both the diagnosis and treatment of PD and related synucleinopathies.

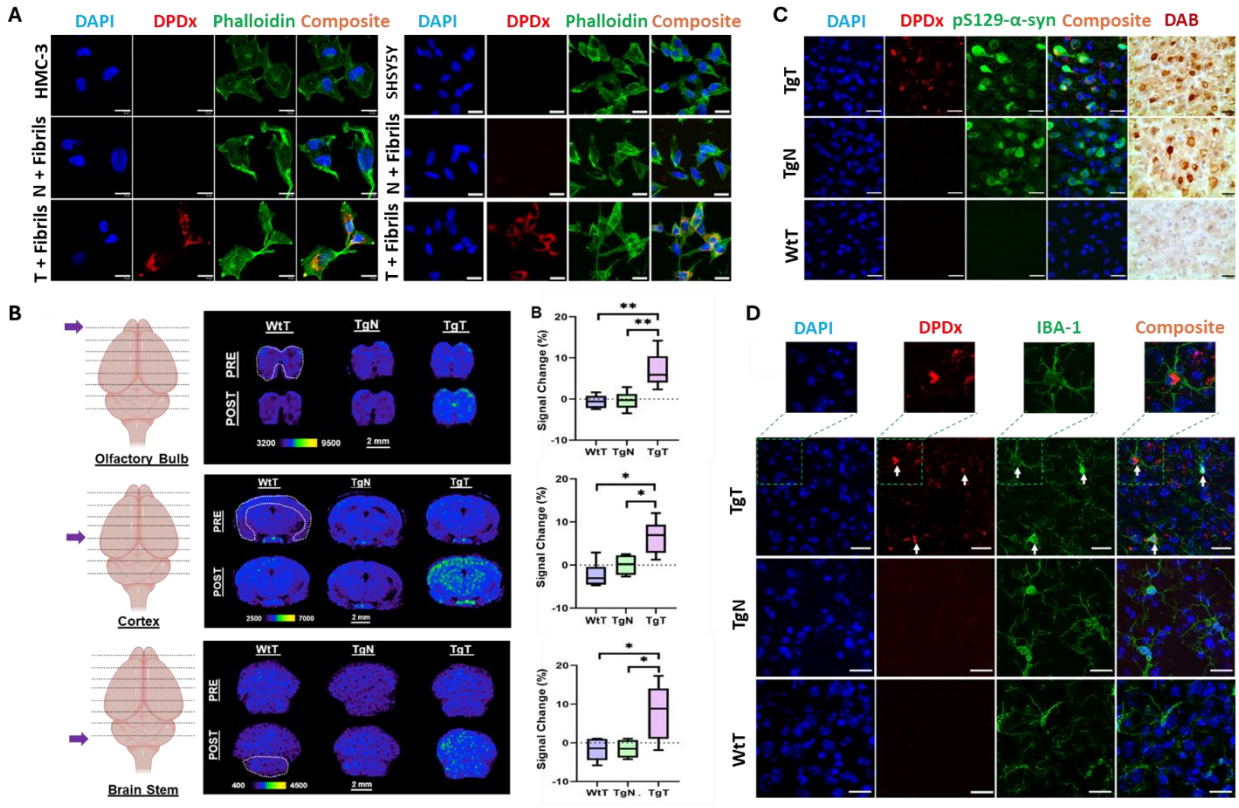


Figure 1. Nanoparticles are taken up by microglia and neuronal cell lines in the presence of α-syn fibrils in vitro and primarily by microglia in vivo following intravenous administration in A53T mice[2]. (**A**) *In vitro* exposure of **DPDx** to differentiated neuronal and glial cell lines in the presence of α-fibrils results in accelerated internalization of **DPDx**. (**B**) *In vivo* T1-weighted MR images pre- and post-contrast administration demonstrated statistically significant signal enhancement in A53T M83 α-syn transgenic mice treated with **DPDx** compared with controls. (**C**) Anti-pSer129-α-syn-stained cortical tissue sections from a 19-month-old **TgT** mouse shows colocalization of rhodamine fluorescence with Lewy pathology. (**D**) Microglia in the entorhinal cortex exhibits *in vivo* uptake of nanoparticles.

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#99

Liposomal Nanoparticle Delivery of mTOR Inhibitors to Alveolar Epithelial Cells to Attenuate Ventilator-Induced Lung Injury

Siying Xu[1,2,3], Qinqin Fei[1,2,3], and Joshua A. Englert[1,2,3]

[1]Division of Pulmonary, Critical Care, and Sleep Medicine, Department of Internal Medicine, and [2]The Dorothy M. Davis Heart and Lung Research Institute, The Ohio State Wexner Medical Center, Columbus, Ohio, USA. 3Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio USA.
Keywords

Acute respiratory distress syndrome (ARDS), mTOR, liposomal nanoparticles, ventilator-induced lung injury (VILI), alveolar epithelial cells (AECs)

Introduction

Acute respiratory distress syndrome (ARDS) patients often require mechanical ventilation (MV) for life support, but MV can cause ventilator-induced lung injury (VILI) (1). The mechanistic target of rapamycin (mTOR) is a central regulator of cell growth, metabolism, and proliferation. Our group previously identified increased mTOR complex 1(mTORC1) activation in alveolar epithelial cells (AECs) as a driver of VILI. Systemic administration of rapamycin, a first-generation allosteric mTOR inhibitor, decreased lung injury when given prior to MV (2). However, systemic delivery of mTOR inhibitors is associated with off target effects (3). Pulmonary delivery of mTOR inhibitors using nanoparticles may improve AEC-targeting, minimize systemic toxicity, and enhance drug solubility. Second-generation active site mTOR inhibitors such as Torin1 and Torin2 potently inhibit mTORC1 and lectin receptors on AECs and can be used for AEC-specific targeting. The goal of our study was to develop liposomal nanoparticles (LNPs) for delivering 2nd generation mTOR inhibitors to AECs to attenuate VILI.

Hypothesis

We hypothesize that LNPs loaded with 2nd generation mTOR inhibitors can mitigate VILI by mTORC1 activation inhibition in lung epithelial cells.

Methods

Torin-loaded lipid nanoparticles (LNPs) were fabricated using thin-film hydration and characterized for particle size, polydispersity index, zeta potential, and encapsulation efficiency. *In vitro*, primary human AECs were cultured on flexible membrane plates and subjected to stretch to simulate VILI. *In vivo* efficacy was assessed using a murine model.

Western blotting was used to evaluate mTORC1 activation by detecting phosphorylated ribosomal S6, a known target of mTORC1, in protein samples isolated from *in vitro* and *in vivo* experiments. To enhance cell-specific delivery, nanoparticles containing a surface ligand for lectin receptors (wheat germ agglutinin (WGA) were formulated and tested in AECs *in vitro* and mice *in vivo*. An *ex vivo* drug release assay was performed to evaluate the release of mTOR inhibitors from both non-targeted lipid nanoparticles.

Results

In vitro, Torin-loaded LNPs treatment suppressed mTORC1 activity in stretched AECs (Fig 1a). Torin-loaded LNPs did not effectively inhibit mTORC1 activation *in vivo* in mice. WGA conjugated LNPs loaded with Torin also inhibited mTORC1 *in vitro* cultured AECs (Fig 1b). *In vivo*, WGA conjugated LNPs loaded with mTOR inhibitors did not inhibit mTORC1 activation in either lung tissue or isolated AECs from spontaneous breathing mice. *Ex vivo* drug release studies indicated that both non-targeted LNPs and WGA conjugated LNPs efficiently released the mTOR inhibitor (Fig 1c and 1d).

Conclusion

Pulmonary delivery of Torin-loaded LNPs effectively inhibited mTORC1 signaling *in vitro*, but not *in vivo*. The lack of *in vivo* efficacy may be related to inadequate dosing or suboptimal drug release at the target site. Future studies will focus on optimization of the nanoparticle formulation to enhance *in vivo* efficacy.

Acknowledgements

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#100

TERT mRNA-loaded lipid nanoparticles reduce neuroinflammation and oxidative stress after traumatic brain Injury

Goknur Kara[1], Morgan Holcomb[1], Anjana Tiwari[2], Hannah Flinn[1], Trinity Eimer[1], Austin Marshall[1], Marissa Burke[1], Peter Park[1], Karem Court[2], John P Cooke[3], Biana Godin[2*], Sonia Villapol[1,4*]

[1]Department of Neurosurgery and Center for Neuroregeneration, Houston Methodist Research Institute, Houston, TX, USA; [2]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA; [3]Department of Cardiovascular Sciences, Houston Methodist Hospital, Houston, TX, USA; [4]Department of Neuroscience in Neurological Surgery, Weill Cornell Medical College, NY, USA.

Keywords: traumatic brain injury, lipid nanoparticles, TERT mRNA, neuroinflammation, nanomedicine.

Introduction

Traumatic brain injury (TBI) is a leading cause of disability and mortality, affecting ~69 million individuals globally and 2.8 million annually in the United States. Beyond acute damage, TBI is now recognized as a chronic condition with long-term neurological consequences, including increased risk of late-onset neurodegeneration [1]. Despite its prevalence, there are no effective clinical therapies to improve long-term outcomes. Telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, maintains telomere length and exerts neuroprotective effects by reducing oxidative stress, apoptosis, DNA damage, and cognitive decline in preclinical models [2]. Nanoparticle-based drug delivery holds significant therapeutic promise for the brain [3].

Hypothesis

We hypothesized that restoring TERT expression using mTERT mRNA-loaded lipid nanoparticles (mTERT-LNPs) would reverse TBI-induced telomere shortening and mitigate neuroinflammation and neurodegeneration, thereby improving recovery following TBI.

Methods

We engineered and characterized mTERT-LNPs. Cytotoxicity was assessed in N2A neuronal cells at 24 and 48h using WST assay. *In vivo*, moderate TBI was induced in mice using a controlled cortical impact (CCI) injury model, followed by systemic intravenous delivery of mTERT-LNPs. Brain accumulation and major organ biodistribution were analyzed with IVIS imaging, and TERT expression, microglial activation, and oxidative stress were assessed by molecular assays.

Results

mTERT-LNPs had uniform nanoparticle size (~100 nm) and >95% mRNA encapsulation efficiency. mTERT-LNPs were non-toxic *in vitro* and localized specifically to injured brain regions *in vivo*. Treatment increased TERT expression and activity in the brain, reduced microglia-mediated inflammation, and attenuated oxidative stress 3 days post-injury, with sex-dependent effects. No detectable side effects were observed.

Conclusions

mTERT-LNPs demonstrated targeted brain delivery, restored TERT activity, and reduced neuroinflammation and oxidative stress after TBI, highlighting their therapeutic potential. These findings support further investigation of TERT mRNA nanotherapy as an innovative approach to improve outcomes in TBI and potentially other neurodegenerative conditions.

Acknowledgements
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#101

Localized Activation of Nrf2 with Antioxidant Response Activating Nanoparticles (ARAPAs) Suppresses Atherosclerotic Plaque Progression

Ana Cartaya#[1,2], Bruno Musetti#[2], Nicholas Buglak[2], Sarah Torzone[2], Geraldine Messinger[2], Danial Babaki[2], Veronica Lisi[3], Edward Bhanson[2], Sophie Maiocchi[2,3*]

[1]Light Microscopy Core Facility, Duke University and Duke University Medical Center, North Carolina, Durham, USA. [2]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, North Carolina, Chapel Hill, USA. 3Department of Biomedical Engineering, Wake Forest University School of Medicine, North Carolina, Winston-Salem, USA.

#co-first

Keywords: Atherosclerosis, Polymeric Nanoparticle, Flash Nanoprecipitation

Introduction

Atherosclerosis is the leading cause of death world-wide with few novel therapies available despite the ongoing health burden. Oxidative stress and inflammation are well-established drivers of atherosclerotic progression; however, the clinical translation of anti-inflammatory, redox-based therapies is lacking. One of the challenges facing redox-based therapies is their targeted delivery to sites of redox dysregulation. A promising class of drug molecules are Nrf2 activators, whereby Nrf2 activation results in the over-expression of several antioxidant and cyto-protective enzymes that may be athero-protective. In prior work, we developed Antioxidant Response Activating Nanoparticles (ARAPas), containing a pharmacological activator of Nrf2, CDDO-Me.1 We demonstrated that these CDDOMe-ARAPas are internalized by macrophages (cells which are highly prevalent in atherosclerotic plaque), and activated Nrf2, suppressing cytokine release by pro-inflammatory macrophages. We also demonstrated that these ARAPas accumulated in atherosclerotic plaque, and activated Nrf2 in murine aorta, whereas a dose-matched administration of CDDOMe had no effect. In the present project, we extend this work to examine whether CDDO-ME ARAPas inhibit atherosclerotic plaque progression in mice.

Hypothesis:

We propose that targeted delivery of pharmacological Nrf2 Activators will inhibit atherosclerotic plaque progression through suppression of inflammation and oxidative stress.

Methods

Generation of nanoparticles: we employed flash nanoprecipitation to generate polymeric nanoparticles of CDDO-methyl (nanomolar pharmacological Nrf2 activator).

Mouse model of atherosclerosis: we employed high-fat diet fed LDLR deficient mice, treated for 8 weeks once weekly with CDDOMe-ARAPas. We used both histology and immunofluorescence of aortic roots to determine atherosclerotic plaque burden and quantify inflammation and markers of oxidative stress. We also employed whole-tissue imaging of the aortic arch with light sheet fluorescence microscopy combined with machine learning to provide unbiased quantification of atherosclerotic plaque.

Results

Treatment with CDDOMe-ARAPas reduced atherosclerotic plaque burden in aortic roots and in the aortic arch by ~40%. Lipid deposition and macrophage infiltration in

atherosclerotic plaque were decreased. Moreover, antioxidant proteins regulated by Nrf2 were increased relative to untreated mice, whereas markers of inflammation (IL1β) and oxidative stress (8-OH-DG, Nitrotyrosine) were reduced.

Conclusion

Localized delivery of pharmacological activators of Nrf2 by CDDOMe-ARAPas suppressed atherosclerotic plaque progression. The local atherosclerotic plaque environment demonstrated reduced inflammation and oxidative stress. Nrf2-activating ARAPas represent a promising targeted therapy to suppress atherosclerotic plaque progression.

Acknowledgements

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#102

Title: Engineering PEGylated Chitosan–Gold Nanoparticles for Radiosensitization and siRNA Delivery to Glioblastoma

Authors: Wesley Chiu; Aria Sabbagh, MD; Yuri Mackeyev, PhD; Sunil Krishnan, MD
Department of Neurosurgery, McGovern Medical School at UTHealth Houston, Houston, TX

Keywords - Gold nanoparticles, siRNA, radiosensitization, glioblastoma

Introduction: Glioblastoma (GBM) is a fast-growing malignancy of the central nervous system with a poor prognosis. Radiation therapy is included in standard treatment after maximally safe surgical resection, yet treatment for these aggressive brain tumors invariably fails locally and at the margins of the radiation field. Inhibiting the radioprotective transcription factor nuclear factor erythroid 2-related factor (Nrf2) via siRNA can increase radiosensitivity in GBM[1], but clinical use is limited by siRNA instability and delivery challenges. The use of gold nanoparticles (GNPs) has been shown to be an effective form of siRNA delivery with low toxicity and ease of synthesis and functionalization. Internalized GNPs also increase free radical generation in cells treated with radiation, thus inherently serving as a form of radiosensitization itself[2]. Chitosan–gold nanoparticles have been investigated as potential siRNA delivery systems[3] owing to their biocompatibility and cationic surface, which facilitates efficient siRNA complexation. This study seeks to develop a GNP delivery platform with PEGylated chitosan to deliver Nrf2-siRNA for potential radiosensitization of GBM.

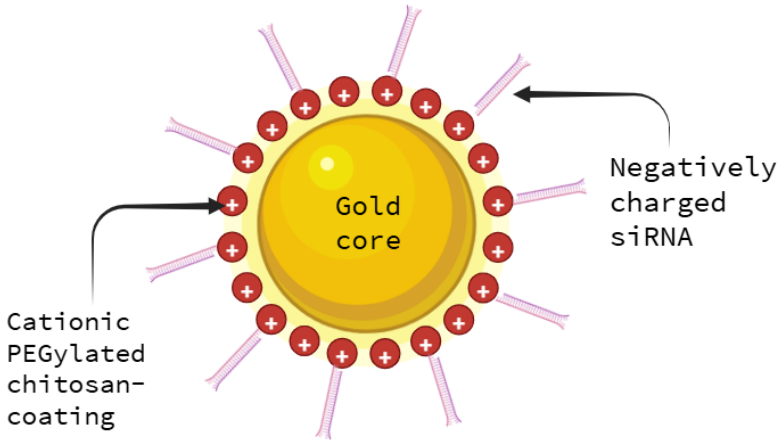
Hypothesis: We hypothesize that PEGylated chitosan–gold nanoparticles can serve as an effective platform for targeted delivery of Nrf2-siRNA to glioblastoma cell lines *in vitro*, leading to Nrf2 knockdown and enhanced sensitivity to radiation.

Methods: Chitosan oligosaccharide was PEGylated by reacting with activated PEG in dimethylformamide using triethylamine as a base, then lyophilized to form a dry powder. PEGylated chitosan served as a reducing and stabilizing agent to form GNPs with a positive surface charge for siRNA binding. Synthesized particles were purified by dialysis membrane, and yield was measured by ICP-MS. SiRNA binding was evaluated by gel retardation assay, while particle size and charge were assessed by dynamic light scattering analysis. *In vitro* siRNA knockdown efficacy was measured on a GL261 mouse glioma cell line using RT-qPCR.

Results: PEGylated chitosan–GNPs formed stable dispersions with an average size of 50 nm and a positive charge conducive to siRNA complexation. Binding studies confirmed efficient loading of Nrf2 siRNA with minimal degradation under assay conditions, indicating the nanoparticles can function as a delivery platform. SiRNA loaded PEGylated chitosan-GNPs incubated with GL261 glioma cells demonstrated effective gene knockdown.

Conclusion: PEGylated chitosan–GNPs with high siRNA binding capacity were successfully synthesized. Further cellular uptake, Nrf2 siRNA knockdown, and radiosensitization studies are underway to assess biological efficacy. This platform shows promise for targeted Nrf2 siRNA delivery to glioblastoma and may serve as a potential radiosensitization strategy.

Graphic:



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#103

Investigating the Functional Contributions of Phospholipids in Selective Organ Targeting Lipid Nanoparticles

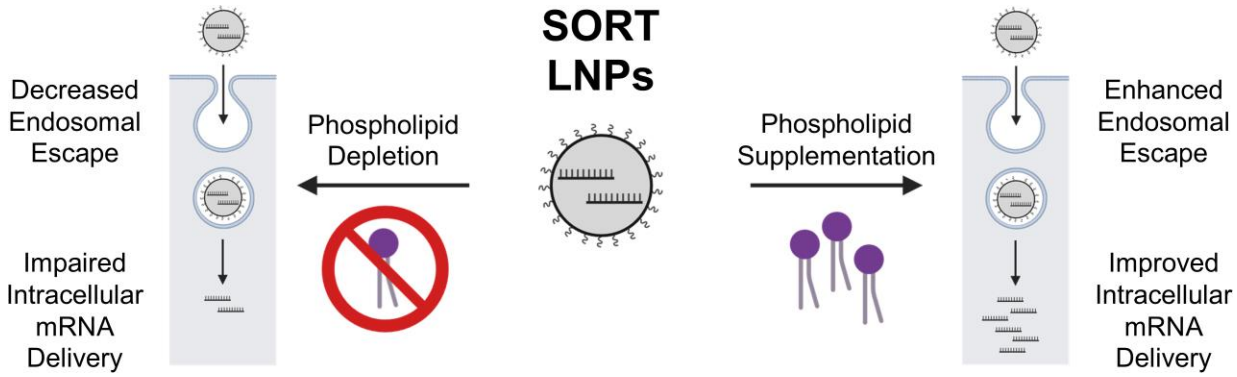
Erick D. Guerrero,[1] Amogh Vaidya,[1] Julien Santelli,[1] Zeru Tian,[1] Gabriela A. Pazzi,[1] Daniel J. Siegwart.[1,*]

[1]Department of Biomedical Engineering, Program in Genetic Drug Engineering, Department of Biochemistry, Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

Abstract

Lipid nanoparticles (LNPs) are among the most clinically advanced platforms for nucleic acid–based therapeutics, establishing mRNA as a next-generation drug for vaccines, protein replacement therapies, cancer immunotherapies, and gene editing. Optimization of LNPs for hepatic and extrahepatic delivery often involves substituting helper lipids or incorporating novel components beyond the conventional four-lipid formulation. While simplifying these systems may ease manufacturing and regulatory evaluation, a deeper understanding of lipid–lipid interactions is critical for improving organ selectivity and transfection efficiency. Among LNP constituents, the role of phospholipids (PLs) in organ-selective delivery remains poorly defined. Here, we systematically evaluate the contribution of PLs within Selective Organ Targeting (SORT) LNPs. We show that PL enrichment enhances cellular transfection efficiency by promoting membrane fusion and endosomal escape. In vivo, PL-containing SORT LNPs significantly increase protein expression following intramuscular administration in mice, whereas moderate PL inclusion is optimal for intravenous delivery. Cryo-electron microscopy further reveals that PL modulation drives distinct structural rearrangements in LNP morphology, which may influence protein corona formation, a key determinant of endogenous targeting.

Collectively, our findings show that PLs function beyond their conventional role as structural “helper lipids.” Instead, they actively support intracellular delivery and shape organ-selective biodistribution through protein corona formation. These results highlight the multifaceted roles of PLs in LNP design and emphasize their importance not only for maintaining structural integrity but also for directing transfection efficiency and organ selectivity. As the field advances, deliberate control of lipid architecture will be essential for engineering next-generation LNPs capable of delivering mRNA-based therapies with greater precision and efficacy.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#104

Regulating Breast Cancer Metastatic Capacity with Molybdenum Disulfide (MoS_2) Nanoparticles

Samantha Foster[1], Kanwar Abhay Singh[2], John Soukar[1], Olajumoke Ogunlusi[3], Christian Nguyen[3], Subiksha Sankar[3], Irtisha Singh[1,4], Tapasree Roy Sarkar[1,3], Akhilesh Gaharwar[1,2]

[1]Interdisciplinary Program in Genetics and Genomics; [2] Department of Biomedical Engineering; [3] Department of Biology; and [4]Department of Cell Biology and Genetics, Texas A&M University

KEYWORDS: Nanoparticles; Molybdenum Disulfide (MoS_2); Breast Cancer; Epithelial-to-Mesenchymal Transition; Metastasis

ABSTRACT: The differentiation of epithelial cells into mesenchymal cells, a process termed epithelial-to-mesenchymal transition (EMT), plays a significant role in the risk and development of metastatic cancers. Epithelial cells exhibit high cell-cell adhesion properties, diminished motility properties, and form tight junctions important for mechanical-based cellular signaling. Conversely, epithelial cells transitioning to a mesenchymal-like phenotype exhibit decreased cell-cell adhesion and increased migratory and metastatic capacity. While EMT plays a role in significant physiological functions such as embryonic development, the shift towards a mesenchymal phenotype within the tumor microenvironment is detrimental, promoting cancer cell extravasation into circulation and subsequent development of the pre-metastatic niche. Therefore, EMT presents a potential therapeutic intervention for preventing the development of metastases. Here, we introduce molybdenum disulfide (MoS_2) nanoparticles as a potential drug-free inhibitor of EMT and metastatic capacity, which is advantageous over traditional chemotherapy-type drugs. This study investigates the impact of MoS_2 nanoparticles on epithelial-to-mesenchymal transition and metastatic capacity in highly aggressive triple-negative breast cancer both *in vitro* and *in vivo*. Evaluations of the effect of MoS_2 on cellular viability revealed that treatment of 25 μg/mL is well within the IC_{80} and was used for the purposes of this study. Treatment of MoS_2 nanoparticles decreased migratory capacity of hMSC and MDA-MB-231 cells in a wound healing assay, comparable to a commercial focal adhesion kinase (FAK) inhibitor (Y15), demonstrating the anti-metastatic motility property of MoS_2. We observed decreased expression of key proteins of focal adhesion site formation such as integrin, vinculin, FAK and smooth muscle actin. Furthermore, the expression of EMT related genes such as *TWIST* and *TGFβR2* are decreased in MoS_2 treated MCF7 and MDA-MB-231 cells after 72h of exposure. Combined, these results indicate that MoS_2 nanoparticles may inhibit EMT progression in a manner comparable to TGFβ1 inhibitors such as RepSox. This effect was further validated with functional 3D mammosphere assays, which indicated reduced clonogenicity in cells following treatment with MoS_2 nanoparticles. *In vivo*, direct injection of MoS_2 into 4T1 mouse mammary tumors significantly decreased primary tumor growth and burden as well as the number of metastatic foci in the lung. Our work demonstrates that MoS_2 nanoparticles inhibit integrin activity as well as the TGFβ signaling cascade pathway, blocking the transition of cells to a mesenchymal-like phenotype. These findings suggest that MoS_2 nanoparticles hold promise for drug-free cancer therapeutic applications in reducing tumor growth and metastatic potential.

#105

Synthesis of RNA-Containing Polymeric Nanoparticles for the Dissolution of NETs

Emily Barnett, Adam Akerman[1], Baisong Lu[2], Nathalie Pinkerton[3], Keith Gagnon[4], Sophie Maiocchi[5]

[1] Department of Surgery, Division of Cardiothoracic Surgery, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7065, USA; [2] Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, USA; [3] Department of Chemical and Biomolecular Engineering, NYU Tandon School of Engineering, Brooklyn, New York, USA; [4]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC; [5] Department of Biomedical Engineering, Wake Forest University School of Medicine, Winston Salem, USA

Keywords – mRNA, Polymeric nanoparticles, drug delivery, NETs

Introduction

Neutrophil extracellular traps (NETs), composed of extracellular DNA and proteins, are formed when neutrophils undergo a specialized form of cell death known as NETosis. Although NETs are recognized as an important part of innate immunity, recent research has demonstrated that dysregulation and excess NET formation contributes to the pathogenesis of diseases such as thrombosis, atherosclerosis, autoimmune diseases, as well as cancer and cancer metastasis[1]. The primary therapeutic used to degrade NETs is deoxyribonuclease 1 (DNase 1), however its therapeutic bioavailability is limited in chronic diseases that require long-term expression due to its short half-life. mRNA based therapeutics allow for continuous translation of encoded proteins, resulting in over-expression between several days, up to a 3 weeks[2]. The purpose of this study is to synthesize mRNA-containing polymeric nanoparticles, capable of overexpressing DNase 1 for the degradation of NETs. A novel, scalable manufacturing method for the synthesis of polymeric nanoparticles (inverse flash nanoprecipitation (iFNP)) will be investigated in its application for the generation of these particles and benchmarked against current lipid nanoparticle formulations.

We **hypothesize** that the use the polymeric nanoparticles for the delivery of mRNA encoding for DNase1 will result in sustained expression of DNase 1 for NET degradation.

Methods

Inverse flash nanoprecipitation (iFNP) was used to synthesize polymeric nanoparticles, consisting of PEG-PDLLA and PDLLA-Poly-L-lysine block-co-polymers +/- yeast RNA or mRNA encoding for green fluorescent protein (mRNA-GFP). Dynamic light scattering was used to characterize size, polydispersity, and charge of individual particle formations. Successful encapsulation of RNA into NPs was confirmed with gel electrophoresis mobility shift assays and release kinetics.

For the generation of DNase 1 mRNA, plasmid DNA (pDNA) was cloned and amplified in E. coli via chemical transformation. Successful synthesis was confirmed through gene sequencing, and mRNA was synthesized from generated plasmids using T7 polymerase in vitro transcription. Successful transcription was confirmed through gel electrophoresis, and protein translation in vitro.

To compare the transfection efficiency of polymeric nanoparticles to commercial lipid based formulations, MC3 mRNA-GFP lipid NPs were generated and characterized with DLS to confirm size, polydispersity, and charge, and ribogreen assays were used to determine encapsulation efficacy. HEK293T cells were transfected with lipid nanoparticles, and transfection efficacy was determined using immunofluorescence staining and flow cytometry.

Results

To confirm RNA compatibility with iFNP, dissolution conditions were optimized to ensure RNA stability throughout NP synthesis (**Figure 1A**). PDLLA-PLL, PEG coated nanocarriers were successfully synthesized using iFNP, resulting in particles ~100 nm in size, a polydispersity index of ~0.3, and a charge of ~0.1 mV, confirmed through DLS characterization (**Figure 1B**). Successful encapsulation of RNA into polymeric nanoparticles was confirmed through agarose gel assay (**Figure 1C**). 24 hr transfection of HEK293T cells, characterized by flow cytometry and immunofluorescence, revealed that mRNA-GFP can successfully be transfected using MC3 lipid nanoparticles.

Conclusions

RNA can successfully be encapsulated into PDLLA-PLL, PEGylated nanoparticles using iFNP. Future studies will investigate their transfection efficiency compared to lipid nanoparticle-based systems.

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#106

Reversal of Senescence in Heart Failure using A Telomerase Circular RNA"

Kiran Kuriakose[1], Rajul Ranka[1], Shuang Li[1], Anahita Mojiri[1], John P. Cooke[1]

[1]Center for Cardiovascular Regeneration, Department of Cardiovascular Sciences, Houston Methodist Research Institute, 6670 Bertner Ave., R10-South, Houston, TX 77030, USA

Keywords – cardiac non-myocytes, circRNA, RNA therapeutics, senescence, telomerase

Introduction

Heart Failure (HF) is a leading cause of morbidity and mortality, with approximately 7 million people affected in the United States alone. Left-ventricular assist device (LVAD) implantation serves as a bridge for patients awaiting heart transplantation, and LVAD support often improves cardiac histology and function. Comparing HF hearts with LVAD-supported HF hearts reveals that cellular senescence, a loss of replicative capacity and cellular energy, drives the myocardium into a failing state. Telomere damage underlies this cellular transition. A promising therapeutic avenue is the use of telomerase, an enzyme capable of repairing genomic DNA and ameliorating the effects of senescence. Prior treatment with mRNA encoding telomerase reverse transcriptase (TERT) substantially reversed endothelial senescence in progeria, an aging model[1,2]. Additionally, we have already shown that Lipid Nanoparticles (LNPs) are an excellent delivery vehicle for TERT mRNA in human skin; we further showed that the mRNA can reduce senescent markers in the skin when exposed to radiation. We are now processing this to optimize the LNP for the heart[3]. Based on our snRNA-seq observations, we choose to study cardiac NMs because of their notable senescence characteristics and score in pre-LVAD compared to post- LVAD cells.

Hypothesis

We hypothesize that the delivery of hTERT by LNPs in cardiac NMs can reverse senescence, potentially through reduction of DNA damage, and restore their replicative capacity.

Methods

We used cardiac NMs from four patients with HF to determine the therapeutic effect of circ-hTERT. The senescent markers in cardiac NMs with LNP alone or LNP-circ hTERT were measured by Immunofluorescence Staining and qPCR. Specifically, we measured β-galactosidase, which increases in senescent cells; CD144, known also as VE-Cadherin, a measure of endothelial integrity; Col1a, a stain for fibroblast detection; 53BP1, for p53 detection, a tumor suppressor protein; and γH2AX, a sensitive biomarker for DNA double-strand breaks. We also looked at replicative capacity of NMs using electric cell substrate impedance sensing assay.

Results

We demonstrate that there is a reversal of senescent characteristics in LNP-circ-TERT treated cells, as opposed to vehicle-treated cells (LNP alone). In vehicle group, CD144 presented weakly;

a similar observation was made in β-galactosidase, γH2AX, and 53BP1 stained cells—the DNA damage was apparent. On the other hand, in circ-hTERT treated cells, CD144 presented abundantly with strong structural and network formations suggesting more vascular cells. It was also observed there was less concentrated islets of γH2AX, and 53BP1 in the nuclei and reduced β-galactosidase staining consistent with the senescence reversal mediated by telomerase. Subsequently, we show that our observations are statistically significant and there is a *decrease* in β-galactosidase, γH2AX, and 53BP1, with a concurrent *increase* in CD144. ECIS assay showed increased replicative potential of treated cells. qPCR results showed decrease in senescent markers p21 and p53, and decrease in inflammatory cytokines IL6 and IL8.

Conclusion(s)

The optimal LNP delivery of TERT successfully demonstrates cellular rejuvenation of cardiac NMs, via a significant drop in DNA damage and senescence markers and concurrent increase in markers for vascular and endothelial health. Moreover, the results of this experiment validate the efficacy of LNP-TERT delivery as a stable and potent vehicle in enzyme delivery and expression. A return to microvascular normalcy boasts profound benefits in patients' capacity for living a healthier lifestyle.

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#107

Overcoming head and neck squamous cell carcinoma chemoresistance with poly(2)oxazoline micelles.

Liubov Palchak[1,2], Xin Li[1], Aimin Peng[1], Alexander V. Kabanov[2]

[1]Department of Biomedical Sciences, Adams School of Dentistry, The University of North Carolina at Chapel Hill, North Carolina, NC 27599, USA; [2]Center for Nanotechnology in Drug Delivery and Division of Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599, USA

Introduction. Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer worldwide, with approximately 54,000 new cases diagnosed in the United States in 2022 (1). Positive treatment outcomes for HNSCC are often hindered by the emergence of chemoresistance in tumor cells. The cornerstone of standard chemotherapy regimens remains cisplatin; however, resistance frequently develops through enhanced DNA interstrand crosslink (ICL) repair. Combining cisplatin with microtubule- targeting agents (MTAs), such as paclitaxel, has been shown to suppress ICL repair and enhance anticancer efficacy (2–3). Despite this promise, the clinical implementation of combination chemotherapy remains challenging due to drug administration scheduling complexities and toxicity concerns.

Hypothesis. Co-loading a reduced dose of cisplatin prodrug (C_6CP) with paclitaxel (PTX) into poly(2-oxazoline) (Pox) amphiphilic micelles, providing sustained release of paclitaxel, may help overcome chemoresistance in HNSCC.

Methods. For micelle preparation, a large-scale thin-film method was employed, followed by tray freeze- drying. The resulting micelles were characterized by determining drug concentrations in POx micelles using reverse-phase HPLC, as well as by assessing size and polydispersity through dynamic light scattering (DLS) and scanning/transmission electron microscopy. In vitro stability and drug-release studies were conducted to evaluate the colloidal properties of the assembled micelles. Cell viability following micelle treatment was assessed in human HNSCC UM-SCC-38 (SCC38) and UM-SCC-11B (SCC11B) cell lines. For in vivo efficacy evaluation, a mouse orthotopic SCC-VII buccal tumor model of HNSCC was used.

Results. We established a rigorous and reproducible procedure for micelle assembly using the POx/PTX/C_6CP platform at weight ratio of 10/3.6/0.6. The resulting micelles exhibited a small size (21.05 ±

1.45 nm) with narrow polydispersity (0.053 ± 0.029). Drug release studies revealed rapid release of the cisplatin prodrug (50% within 2 hours and complete release within 25 hours) and slower release of paclitaxel (50% within 17 hours and complete release after more than 72 hours). Stability studies showed that the micelles remained stable for up to 5 hours at room temperature, 4 days at 4 °C, and 8 days in diluted form. Cell viability assays demonstrated reduced IC_{50} values for the POx/PTX/C_6CP formulation in both SCC11B and SCC38 cells, outperforming the free drug combination. In vivo efficacy testing using an orthotopic SCC-VII buccal tumor model showed that treatment with POx/PTX/C_6CP significantly suppressed tumor growth compared with the free drug combination. Excised tumors from the POx/PTX/C_6CP group were consistently smaller and lighter. Moreover, body weight remained stable, and no toxic events were observed in the treatment group.

Conclusions. A biocompatible amphiphilic poly(2-oxazoline) platform enabling rapid release of a cisplatin prodrug and sustained release of paclitaxel offers therapeutic benefit against cisplatin-resistant HNSCC through microtubule-targeted intervention.

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#108

Triple negative breast cancer cells targeting based on nanoparticles with programmable RNA-responsive elements

Thy Nguyen[1], Ricardo Noriega[2], Vrutant Shah[2], Eric Chau[1], Kristopher Brannan[2], Biana Godin[1]

Houston Methodist Research Institute
(1) Department of Nanomedicine, (2) Center for RNA Therapeutics

Introduction: Triple-negative breast cancer (TNBC) represents 15-20% of all breast cancer diagnoses but contributes disproportionately to cancer mortality due to its aggressive biological behavior and limited therapeutic options. Unlike hormone receptor-positive or HER2-positive cancers, TNBC lacks targetable surface receptors, leaving patients dependent on chemotherapy regimens that frequently fail due to drug resistance and tumor heterogeneity. Current therapeutic approaches targeting surface receptors are fundamentally limited in TNBC due to the absence of these targetable markers, creating an urgent need for novel therapeutic strategies. The intracellular molecular landscape of TNBC cells presents unique opportunities for precision targeting that remain largely unexploited. TNBC cells exhibit distinctive transcriptional reprogramming that generates specific RNA signatures, including overexpression of RNA-editing enzymes like ADAR1 and the presence of unique fusion transcripts that can serve as therapeutic triggers. This reprogrammed transcriptomic environment creates an opportunity for developing "smart" therapeutics that respond specifically to the molecular state of cancer cells while remaining inactive in healthy tissues. Programmable RNA sensors (RNAsen) represent an emerging class of precision therapeutics that can detect specific intracellular molecular signatures and respond with targeted therapeutic. We **hypothesize** that RNAsen combining linear ADAR-mediated sensing mechanisms and lipid nanoparticles can achieve specific recognition and therapeutic targeting of TNBC cells in vitro and in vivo.
Methods: ADAR1-responsive oligonucleotides (reprogrammable ADAR sensors, or RADARs) were designed as potential diagnostic and therapeutic tools against TNBC cells. TNBC-specific RNA sensing endogenous IGFBP3 or COL1A2-triggered activation of payload expression was assessed by transfecting ADAR expressing TNBC cells with either IGFBP3 or COL1A2 RADARS with GFP payload or caspase-9, the “suicide gene” in a single culture. Specificity was then assessed transfecting the same RADARS into co-culture with MCF10A and TNBC cells. The RNAsen were further encapsulated in lipid nanoparticles (LNPs). For *in-vivo* assessment, an orthotopic syngeneic model of TNBC (4T1 in Balb/C mice) was created and the RNAsen expressing luciferase when activated by ADAR1 in TNBC cells were injected intra-tumorally. Luciferase expression was further monitored at 24 and 48h.
Results: Increased reporter (GFP and/or luciferase) expression from mRNAs sensing endogenous transcripts of both IGFBP3 or COL1A2 RADARSR designs upregulated in 4T1, BT-549 and Hs 578T TNBC cells. Specific cell-type fluorescence expressions were increased in TNBC (MDA-MB-231) cells vs. non-cancerous breast epithelium cells (MCF10A) grown in co-culture. IGFBP and COL1A2 RADARS designed with the caspase-9 (“suicide gene”) payload, exhibited lower cell viability when transfected into TNBC cell lines (MDA-MB-231, Hs 578T, ad BT-549) than in MCF10A. In vivo study confirmed that LNPs with RNAsen enabled expression of luciferase following activation in the tumor.
Conclusion: The studies demonstrate the possibility for a novel targeting mechanism using LNPs with programmable mRNA platforms when surface receptors are unavailable. Testing of the proposed programmable nanotherapeutics in metastatic models of TNBC is underway.
Acknowledgments: The Cancer Prevention and Research Institute of Texas (CPRIT) provided the funds necessary to realize this project.

#109

SIRPα-siRNA Lipid Nanoparticles to Disrupt CD47- SIRPα Signaling Axis in Metastatic Ovarian Cancer Models

Eric Chau[1], Sabrina Vandenheuvel[2], Cailin O'Connell[1,2], Karem A. Court[1], Shreya Raghavan[1,2], Aparna Kamat[3,4,5], Biana Godin[1,2, 4]

[1]Department of Nanomedicine, Houston Methodist Research Institute
[2]Department of Biomedical Engineering, Texas A&M University
[3]Division of Gynecological Oncology, Houston Methodist Hospital
[4]Department of Obstetrics and Gynecology, Houston Methodist Hospital
[5]Houston Methodist Neal Cancer Center, Houston Methodist Hospital

Purpose: The 5-year survival of patients with ovarian cancer (OvCa) liver metastases (OCLM) is < 30% due to limited effective therapeutic options. Macrophages (Mϕ) represent a major cell population in OCLM microenvironment, which promotes tumor growth/invasion and enhances chemo-resistance. OvCa cells express surface immunoglobulin CD47 that binds to Mϕ signaling receptor protein-α (SIRPα) suppressing their phagocytic functions and enhancing pro-tumoral capabilities. Disruption of the CD47-SIRPα signaling axis presents a highly relevant Mϕ-targeted immunotherapy strategy for OCLM in conjunction with conventional chemotherapies. We **hypothesize** that disruption of the CD47-SIRPα signaling axis can decrease the metastatic potential of OvCa and contribute to increased chemotherapy efficiency.

Methods: Expression of CD47 and SIRPα was confirmed in metastatic OvCa clinical samples. To disrupt the CD47-SIRPα axis, SIRPα siRNA lipid nanoparticles (LNP) were designed. SIRPα siRNA LNP were characterized for size, zeta potential, encapsulation efficiency and particle concentration by Dynamic Light Scattering, RiboGreen assay and NanoSight, respectively. *In vitro* biocompatibility and cellular uptake of LNP were assessed in two- and three-dimensional cultures, containing homo- or hetero-cultures of OvCa cells (OVCAR3, high grade serous OvCa) and Mϕ (THP-1 derived monocyte). Efficacy in reducing resistance to carboplatin was tested in hetero-spheroids treated with either Carboplatin (0-1000 μM) with and without SIRPα siRNA LNP (siRNA 0-50 nM) for 48 hours. RT-qPCR was performed to quantify expression of CD47 and SIRPα as well as metastasis-indicating genes in OvCa cell models. *In vivo* studies were conducted in metastatic OvCa syngeneic model (C57L/J mice inoculated with 5M ID8-luc cells IP). The therapeutic effects were assessed through tumor burden IVIS, SIRPα knockdown in liver samples, and organ IHC/IF.

Results: The CD47-SIRPα targeting approach for OvCa was validated with increased expression of CD47 and SIRPα in metastatic versus normal ovarian clinical tissue samples. SIRPα siRNA LNP was successfully formulated with narrow size distribution (polydispersity index <0.1) and diameter within 64-90nm range. The formulation showed consistent size distribution, zeta potential, and concentration across the evaluated time period. RNA content showed >95% encapsulation efficiency and minimal degradation in non-lyophilized systems up to day 12 of cold storage. The siRNA LNP showed high in vitro biocompatibility and efficient cellular uptake in cultures. Mϕ contributed to carboplatin chemoresistance in hetero-spheroids (1.7-fold more resistant than OvCa mono-spheroids). Our results indicate that siRNA nanoparticles treated with OvCa/ Mϕ hetero-spheroids yielded in 50% silencing of SIRPα expression. Disruption in Mϕ SIRPα signaling via SIRPα siRNA LNP inhibited OvCa cell proliferation. *In vivo*, SIRPα siRNA LNP and carboplatin treatment in ID8 metastatic OvCa murine model showed significant decrease in luminescence signal and proliferation index, when comparing to controls.

Conclusions: The study confirms the ability to design and generate LNP systems that target and disrupt the CD47-SIRPα signaling axis through siRNA for advanced OvCa therapy. Efficacy in disruption of the CD47- SIRPα axis has been confirmed in vitro in hetero-spheroids and in vivo in metastatic OvCa models. This approach can represent a potential novel strategy for Mϕ targeted immunotherapy to enhance the efficacy of chemotherapy regiments in advanced OvCa, reducing tumor burden and improve prognosis.

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#110

Fluorinated Lipid Probes Unveil the Molecular Basis of Tail-Chain Regulation in Lipid Nanoparticles

Haonan Huo[1], Jihang Zhai[2], Jingjiao Li[1], Ning Chen[3], Xiao He[4], Jiafei Mao[1], Xueguang Lu[1]

[1] Institute of Chemistry, Chinese Academy of Sciences, [2] East China Normal University, [3] Beijing University of Chemical Technology, [4] Chongqing Institute of East China Normal University

Keywords
Fluorine • Lipid Nanoparticle• mRNA • NMR spectroscopy • Structure-activity relationships

Introduction

Lipid nanoparticles (LNPs) are essential carriers for mRNA therapeutics, as exemplified by COVID-19 vaccines. Ionizable lipids (ILs) play a critical role in mRNA encapsulation and release, but the molecular mechanisms by which IL tail chemistry regulates LNP function remain poorly understood. While cryo-EM and small-angle neutron scattering (SANS) provided nanoscale morphological insights, atomic-resolution interactions of ILs with cholesterol and other LNP components are largely unknown.

Hypothesis

We hypothesize that the introduction of a cis double bond into ionizable lipid tails reconfigures molecular packing within LNPs. By incorporating fluorine atoms and applying ^{19}F solid-state NMR (SSNMR), we aim to directly probe and validate these cis double bond–induced molecular interactions.

Methods

We synthesized a library of fluorinated SM102 analogues (F-SM102s) with varied chain lengths, degrees of fluorination, and unsaturation. LNPs were formulated with F-SM102s and characterized by dynamic light scattering (DLS) and *in vivo* luciferase mRNA expression. Ultrafast magic angle spinning (VFMAS) ^{19}F solid-state NMR and ^{1}H–^{19}F HOESY spectra were acquired to probe molecular interactions. All-atom molecular dynamics (MD) simulations complemented experimental findings. Biophysical and functional assays included DPH fluorescence anisotropy for membrane fluidity, hemolysis for membrane disruption, flow cytometry, and confocal microscopy for cellular uptake and endosomal escape.

Results

F-SM102s formed stable LNPs with efficient mRNA encapsulation. *cis*-C9F13-LNP achieved up to 2.3-fold higher *in vivo* luciferase expression than C9F13-LNP. ^{19}F SSNMR revealed that linear C9F13 interacts strongly with cholesterol, while *cis*-C9F13 disrupts such packing, favoring IL self-association. MD simulations confirmed conformational folding induced by *cis* double bonds, leading to reduced cholesterol

contacts and enhanced headgroup proximity. Biophysical assays showed lower DPH anisotropy and increased membrane disruption for *cis*-C9F13-LNP, correlating with more efficient endosomal escape and higher mRNA release.

Conclusions

This study demonstrates that a single cis double bond in fluorinated IL tails reconfigures molecular packing and cholesterol interactions, producing more dynamic and fluid LNPs with improved mRNA delivery. Fluorinated ILs serve both as functional delivery agents and powerful ^{19}F NMR probes for mechanistic dissection of LNPs. These insights provide a framework for rational IL design in next-generation mRNA nanomedicine.

Acknowledgements

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#111

AMPING: Antibody-Microbubble-Mediated Peptide-Based Immune PrimiNG

Shea Garland[1], Seongdong Jeong[2], Thomas Gallup[2], Adam Woodward[1], Catherine Valadez[3], Christien Carter[3], Glen Liszczak[3], Wen Jiang[2], Jacques Lux[1,4]

[1]Department of Radiology, Translational Research in Ultrasound Theranostics (TRUST) Program, University of Texas Southwestern Medical Center; [2]Departments of Neurosurgery and Radiation Oncology, The University of Texas MD Anderson Cancer Center; [3]Department of Biochemistry, University of Texas Southwestern Medical Center; [4]Department of Biomedical Engineering, University of Texas Southwestern Medical Center

Introduction & Hypothesis

Malignant tumors arise from mutations that produce altered proteins, generating novel peptide antigens. These can be presented by antigen-presenting cells (APCs) via the major histocompatibility complex (MHC), triggering an adaptive immune response via $CD8^+$ and $CD4^+$ cytotoxic T lymphocytes (CTL). This process is the cornerstone by which the immune system eradicates malignant cells. Immune checkpoint inhibitors (ICIs) enhance this response, however, tumors with low mutational burden are often not responsive to ICI treatment.[1] This project aims to develop a cancer vaccine to “heat up” such tumors to sensitize them to immune checkpoint blockade.

To do this, tumor-specific antigens (TSAs) will be conjugated to APC-targeted microbubbles (MBs) via a reducible disulfide linkages. When exposed to ultrasound, MBs oscillate and collapse to open transient pores in the membrane of the targeted cells to deliver their payload through a process termed sonoporation. The reducing intracellular environment will then cleave the disulfide bonds to release the antigens and promote their presentation by APCs (**Fig. 1**).[2]

Methods

The $OVA_{257-264}$ peptide (SIINFEKL) was synthesized with an N-terminal cysteine (CSIINFEKL) for conjugation to DSPE PEG(2k) PDP via disulfide exchange. Peptide conjugation and release was confirmed via fluorescence microscopy and flow cytometry. To confirm CSIINFEKL presentation on MHC-I, bone marrow-derived macrophages (BMDMs) were pulsed with peptide, stained with 25-D1.16, and evaluated by flow cytometry. MBs composed of 90% DSPC and 10% DSPE PEG(2k) PDP were then formulated and conjugated with CSIINFEKL and aCD11b, incubated with BMDMs, and sonoporated with a 15 mM transducer. After 16 h, antigen presentation and OT-I T cell proliferation were assessed by flow cytometry.

Results

Co-localization of fluorescent signal on the shell of the MB was observed by fluorescent microscopy suggesting successful conjugation of the peptides on the MBs’ shell. Decay in the fluorescent signal was observed by flow cytometry when fluorescein-labeled peptide-conjugated MBs were exposed to DTT at 37 °C. CSIINFEKL induced more antigen presentation than native $OVA_{257-264}$ peptide. Furthermore, antigen presentation increased by >5-fold using targeted MBs, suggesting the efficiency of sonoporation as a method to deliver peptide antigens to APCs. Compared to free peptide, the AMPING platform increased presentation 6.5-fold and drove the greatest OT-I proliferation, indicating strong in vivo potential.

Conclusions

This work provides the foundation to develop a platform for the delivery of neoantigens to APCs for personalized cancer vaccines.

If successful, this platform will be combined with STING activators to both activate the STING pathway and enhance antigen cross-presentation to improve anticancer immune response. In vivo studies are in progress to determine efficacy against SB28-OVA, an OVA protein-expressing murine orthotopic glioblastoma model.

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Figure

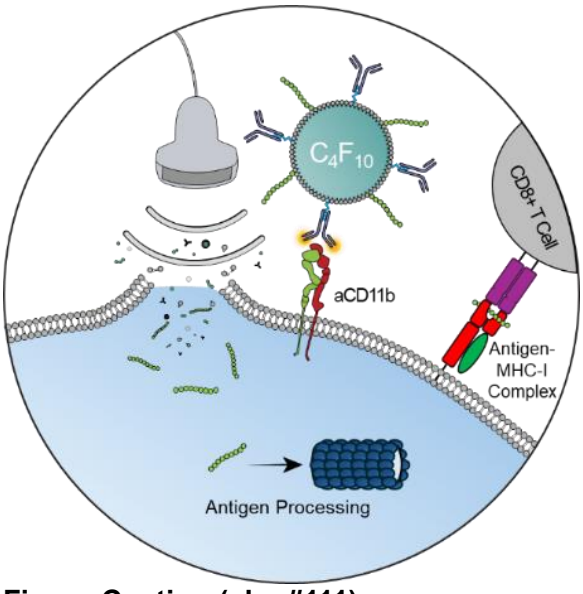

Figure Caption (abs #111)
High-intensity ultrasound bursts MBs and delivers antigen-lipid conjugates to the cytosol. Upon exposure to the reducing environment, the peptide is cleaved from the lipid and processed in the cell before being presented by the MHC-I.

#112

Improving shelf life of nanocomplex-conjugated microbubbles for image-guided drug delivery

Anbarin Khalid [1,2], Sina Khorsandi [1], Nazia Hafeez [1,2], Adam Woodward [1], Shea Garland [1], Jacques Lux [1,2]

[1]Department of Radiology, Translational Research in Ultrasound Theranostics (TRUST) Program, UT Southwestern Medical Center

[2]Department of Biomedical Engineering, UT Southwestern Medical Center

Keywords: ultrasound, sonoporation, microbubble, freeze-drying, image-guided therapy

Microbubbles (MBs) are ultrasound (US) contrast agents that have been used in the clinic for more than three decades. In recent years, the use of MBs as vehicles for drug delivery has been explored due to their response to ultrasound and ability to transiently permeabilize cell membranes, a process known as sonoporation. Our lab recently developed MUSIC (microbubble-assisted ultrasound-guided immunotherapy of cancer), in which nanocomplex-conjugated MBs (ncMBs) effectively deliver the cyclic dinucleotide cGAMP into antigen-presenting cells (APCs), activating the cytosolic stimulator of interferon genes (STING) pathway and eliciting potent antitumor immune responses in murine breast cancer models.[1] The efficacy of the ncMBs were further validated in aggressive murine models of melanoma.[2]

However, the clinical translation of therapeutic MBs remains limited by their short shelf life as lipid-shelled MBs typically remain stable only hours to days post-production. Freeze-drying has emerged as a promising solution for long-term storage of MBs while maintaining echogenicity and payload stability.[3] We hypothesize that freeze-drying ncMBs will extend their shelf life and preserve their acoustic properties as well as their targeting and therapeutic functionalities post-reconstitution. Storage of such therapeutic MBs as a dry powder would enable scalable production and facilitate clinical applications.

ncMBs were formulated with a perfluorobutane (PFB) gas core and a phospholipid shell containing DSPC and DSPE-PEG-maleimide. To impart MBs with drug loading and targeting capabilities, cationic spermine-modified dextran and CD11b-targeting antibodies (aCD11b) were thiolated and conjugated onto the MB shell to encapsulate for specific delivery in $CD11b^+$ APCs. Following formulation, ncMBs were freeze-dried using 10% sucrose as a cryoprotectant. Following lyophilization, ncMBs were stored in the freezer in a powder form with a PFB headspace. ncMBs were reconstituted simply by adding PFB saturated water and mixing using various methods. ncMBs' size distribution, zeta potential and echogenicity were assessed before and after freeze-drying and reconstituting. We also evaluated the targeting ability and ultrasound-aided cytosolic delivery capacity of reconstituted MBs by applying US at 2 W/cm^2, 50% duty cycle for 1 minute on bone marrow-derived macrophages at different dosages and measured STING activation.

This work intends to provide a robust method to freeze-dried targeted therapeutic MBs can retain their acoustic responsiveness, targeting ability, and structural stability after long-term storage following simple reconstitution. If successful, this strategy would allow scalable off-site production and easy transportation of MBs from bench to bedside, which provides the foundation to develop point-of-care application of therapeutic MBs.

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#113

Title: Porous liquid: Integrating Porosity and Fluidity for Enhanced Drug Delivery

Authors: Xueying Ge[1], Yongbin Liu[2], Junhua Mai[2], Shengqian Ma[3,*] and Roderic I. Pettigrew[1,*]

Affiliations:

[1]School of Engineering Medicine/ENMED, Texas A&M University and Houston Methodist Hospital, Houston, Texas 77030, USA

[2]Department of Nanomedicine, Houston Methodist Academic Institute, Houston, TX 77030, USA

[3]Department of Chemistry, University of North Texas, Denton, TX 76201, USA

Keywords: Porous Liquids, Drug Delivery Systems, Hydrophobic Drugs; Extended Circulation, Tumor Deposition

Introduction: Over 70% of approved drugs are hydrophobic and suffer from poor bioavailability, posing a major barrier to therapeutic efficacy. This is largely due to the entropic penalty of disrupting water's hydrogen-bond network and the high lattice energy of these drugs. To address these challenges, three key strategies have emerged: (1) organic cosolvents like dimethyl sulfoxide (DMSO) or ethanol can disrupt hydrogen bonding but pose toxicity risks and lack controlled release; (2) solid nanocarriers (e.g., silica, polymers, lipid-based systems, and porous frameworks) use porous structures to enable high drug loading and controlled release, while shielding hydrophobic drugs from aqueous environments, yet they are rapidly cleared by the mononuclear phagocyte system when administered intravenously; (3) ionic liquids (ILs) improve solubility by disrupting hydrogen bonding and weakening lattice energy through their polarity and salt-like properties, and IL-coated nanoparticles have shown extended circulation. However, ILs lack porosity, limiting drug-loading capacity and release control. A platform that integrates high loading/controlled release with extended circulation remains needed.

Hypothesis: We hypothesize that porous liquids with permanent porosity can serve as a novel hydrophobic drug delivery platform by uniting the high drug loading and controlled release of porous solids with the solubility and bioavailability advantages of liquid systems, thereby improving delivery efficiency and therapeutic outcomes.

Methods: We synthesized a type 1 silica-based porous liquid (HMOV-liquid) by modifying hollow silica nanoparticle cores into phenylene-bridged hollow mesoporous organosilica vehicles (HMOVs) and linking them to poly(ethylene glycol)-tailed sulfonate salt (PEGS) via a polymerized ionic liquid. Doxorubicin (DOX) and 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-Chlorobenzenesulfonate salt (DiD), chosen for their aromatic π–π stacking interactions with HMOV-liquid, were used as model compounds. pH-dependent DOX release was measured under physiological (pH 7.4) and acidic (pH 5.0) conditions. Macrophage phagocytosis of porous liquid versus solid carriers was quantified by flow cytometry and fluorescence microscopy. Pharmacokinetics, biodistribution, and acute toxicity were evaluated in nude mice following intravenous injection, with IVIS imaging and blood analyses over two weeks.

Results: Our uniquely designed porous liquids merge the payload capacity and controlled release of permanent pores with the biotransport advantages of a liquid continuum. HMOV-liquid achieved: ~90 wt% DOX loading; burst-free, pH-responsive release (~6.8% at pH 7.4 vs ~71% at pH 5.0 over 144 h); >10-fold lower macrophage uptake at 0.5 h than solid HMOVs; ~2-fold reduced hepatic trapping and 15.9 ± 0.6% injected dose remaining in blood at 24 h. Notably,

HMOV-liquid reached a tumor-to-liver ratio of 3.37 compared with ~0.3 for solid HMOVs and <0.5 for PEGylated liposomes, achieved without the need for added targeting ligands.

Conclusion: We report the first porous-liquid drug-delivery platform. HMOV-liquid mitigates the rapid clearance that limits solid nanocarriers and, unlike ionic liquids, provides permanent porosity for high hydrophobic-drug loading with programmable release. Ongoing studies investigate why prolonged circulation increases tumor deposition. Thus, porous liquids replace long-standing tradeoffs with simultaneous benefits in solubility, retention, loading, and circulation, enabling tunable, generalizable formulations.

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