18th International Symposium on Recent Advances in Drug Delivery Systems

A TRIBUTE TO THE LATE SUNG WAN KIM

February 22nd—24th, 2022

Salt Lake City, Utah USA

Hosted by:

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Presented by:

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WELCOME

It is our great pleasure to welcome you to the 18th International Symposium on Recent Advances in Drug Delivery Systems. This event is a tribute to the late Sung Wan Kim – a giant in the area of drug and gene delivery and the founder, with Jim Anderson, of this meeting. The first International Symposium on Recent Advances in Drug Delivery Systems took place in 1983 in the Yardley Hotel in Park City, Utah. From the second meeting it was moved to Salt Lake City. This is one of the earliest and greatest conferences in the area of drug delivery, always attracting the top scientists in the field. Oral presentations were by invitation only and the organizers always devoted significant effort to correctly represent the best science in the drug delivery area. From the 6th symposium onward, posters were included and this enhanced the participation of young scientists. Numerous coworkers from the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah contributed to the organization of the meeting. From 1991 – 2005 Henry Kopeček joined Sung Wan Kim and Jim Anderson as organizer. Starting with the 13th symposium in 2007 David Grainger, You Han Bae, and Kinam Park took over the organization. The last meeting (17th) took place in 2015. Sung Wan Kim would be happy to know that, after a short lull, the meeting has resumed.

Sung Wan's pioneering research in biomaterials and drug delivery has been inspiring for the scientific community. In particular, his contributions to the science of hydrogels, biodegradable drug conjugates, stimuli-sensitive polymers, and polymer-based gene-delivery systems were truly visionary. These interdisciplinary research results from his laboratory were not only translated into the clinics but also created scientific foundations that played an influential role in facilitating further inventions from scientists around the world.

The organizers look forward to excellent presentations that will demonstrate the high level of science in drug delivery research. We strongly believe that this is the proper tribute to Sung Wan Kim.

Our sincere thanks to the sponsors who made the organization of this conference possible. In particular we would like to extend our appreciation to the Sam Yang Corporation and the Kim family for their generous support as well as the many alumni and friends of Sung Wan Kim.

We hope that you enjoy the conference and your stay in Salt Lake City, including skiing in the canyons, as skiing has always been a popular part of the meeting. For those that cannot attend in person, your virtual participation is appreciated.

Organizing Committee

Eighteenth International Symposium

On

Recent Advances in Drug Delivery Systems

A tribute to the late Sung Wan Kim

February 22-24, 2022

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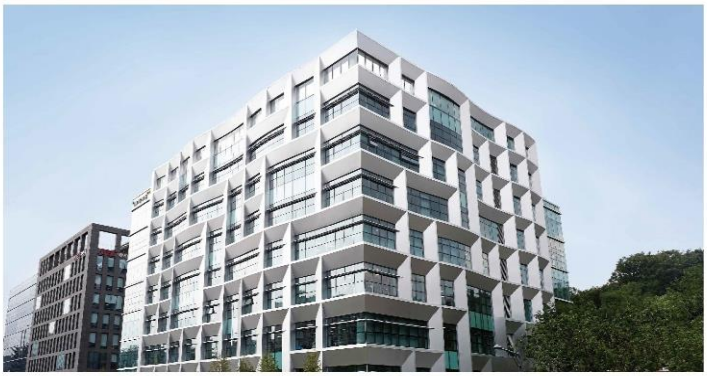

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ESTABLISHMENT
2017

PRODUCTS
Injections, API

LOCATION
Daejeon, Korea

CERTIFICATIONS
EU GMP, PMDA(Japan) GMP, KGMP(Korea)

• Medical Devices Plant

ESTABLISHMENT
1996, 2019 (Full operation expected by 2022)

PRODUCTS
Sutures, Hemostats, Patches

LOCATION
- Daejeon, Korea
- Godoll, Hungary

CERTIFICATIONS
KGMP, CE Mark, ISO13455(Medical Devices) KGMP(Pharmaceuticals)

• Samyang Biopharm USA Inc.

ESTABLISHMENT
2018

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LOCATION
Cambridge, MA, USA

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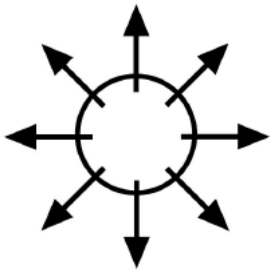
Leading scientists in the field of drug delivery contributed to these special issues of Advanced Drug Delivery Reviews dedicated to Prof. Sung Wan Kim when he suddenly passed before the completion of these issues.

Scan the QR codes in front of the banner above, to access all three issues

TECHNICAL PROGRAM



18th International Symposium on Recent Advances in Drug Delivery Systems

Program Schedule

Tuesday, February 22nd, 2022

Time	Event	Location
6:00 pm	Welcome Reception	Summit View Room (2nd floor)

Wednesday, February 23rd, 2022

Time	Event	Location
7:00 am	Registration	Bonneville Ballroom Atrium (1st floor)
	Breakfast	

Welcome/Introductory Remarks by

Time	Speaker	Location
8:00 am	**Erin Rothwell** Vice President for Research University of Utah **Randall Peterson** Dean, College of Pharmacy **Hamid Ghandehari** Chair, Department of Pharmaceutics and Pharmaceutical Chemistry	Bonneville Ballroom (1st floor)

Session 1 Moderator: David Grainger

Time	Speaker	Title
8:15 am	**James Anderson** Case Western Reserve University	The Sung Wan Kim I Knew
8.35 am	**Jindřich Kopeček** University of Utah	Smart Nanomedicines Based on Receptor Crosslinking
8:55 am	**Nicholas Peppas** The University of Texas at Austin	Cationic Nanogels for Oral Targeted siRNA Delivery to Macrophages for Treatment of Inflammatory Bowel Diseases
9:15 am	**Helen Cho** Samyang Holdings	Preclinical Development of Tissue-Selective Polymer-Lipid Based Nanoparticle Formulations for the Delivery of mRNA Vaccine and Therapeutics
9:35 am	**Shreya Goel** University of Utah	Multiscale Imaging in Nanomedicine: A Value Proposition
9:55 am	Coffee break	

Session 2 Moderators: Carol Lim and Yue Lu

Time	Speaker	Title
10:10 am	**Jan Feijen** University of Twente	In Memory of Prof. Dr. Sung Wan Kim
10:30 am	**Wim Hennink** Utrecht University	Stability of Drug-Loaded Polymeric Micelles
10:50 am	**Suzie Pun** University of Washington	VIPER: A Virus-Inspired Polymer for Endosomal Release
11:10 am	**Jinming Gao** UT Southwestern Medical Center	Nano-Immuno-Oncology: Harnessing Molecular Cooperativity for Cancer Immunotherapy
11:30 am	**Tony Mikos** Rice University	Injectable and 3D-Printable PNiPAAm-Based Hydrogels for Tissue Engineering
11:50am	**Ram Mahato** University of Nebraska	Polymeric Nanomedicine of Multiple Small Molecule Drugs for Treating Medulloblastoma
12:10 pm	**Lunch**	
	Poster session	

Session 3 Moderator: Mingnan Chen

Time	Speaker	Title
1:10 pm	**Christopher Porter** Monash Institute of Pharmaceutical Sciences	Triglyceride-mimetic Prodrugs to Enhance Lymphatic Transport
1:30 pm	**Byeongmoon Jeong** Ewha Womans University	Biodegradable Thermogels for 3D Cell Culture
1:50 pm	**Yong Hee Kim** Hanyang University	The Late Professor Sung Wan Kim-inspired Oligopeptide Complex for Targeted Non-viral Gene Delivery
2:10 pm	**Yu-Kyoung Oh** Seoul National University	Nanomaterials for Modulation of Tumor Immune Microenvironments
2:30 pm	**Hideyoshi Harashima** Hokkaido University	Multifunctional Envelope-type Nano Device from Controlled Intracellular Trafficking to Clinical Application for Nanomedicines
2:50 pm	**Youngro Byun** Seoul National University	The Strategy to Treat pan-KRAS Mutant Cancers by Using Albumin Binding Caspase-3 Cleavable Peptide-drug Conjugate
3:10 pm	Coffee break	

Session 4 Moderator: Makoto Kondo

Time	Speaker	Title
3:25 pm	**Teruo Okano** University of Utah	Juvenile Chondrocyte Sheet-based Allogeneic Cartilage Regenerative Therapy
3:45 pm	**Kazunori Kataoka** Kawasaki Institute of Industrial Promotion	Engineered Nanosystems and Nanoconjugates with Smart Functionalities for Targeted Therapy of Intractable Diseases
4:05 pm	**Chae-Ok Yun** Hanyang University	Oncolytic Adenovirus: New Opportunity for Targeted Cancer Treatment
4:25 pm	**Akihiko Kikuchi** Tokyo University of Sciences	Stimuli Responsive Hydrogels Degradable Under Cancer Environment
4:45 pm	**Zhiyuan Zhong** Soochow University	Bioresponsive Polymersomes for Cancer Therapy and Immunotherapy
5:05 pm	**Poster session**	
7:15 pm	**Banquet**	

Thursday, February 24th, 2022

Time	Event	Location
7:00 am	*Breakfast*	Bonneville Ballroom Atrium (1st floor)

Session 5 Moderator: You Han Bae

Time	Speaker	Title
7:45 am	**Ick Chan Kwon** Korea Institute of Science and Technology	Activatable Imaging Probe for Receptor-Ligand Binding
8:05 am	**Doo Sung Lee** Sungkyunkwan University	Degradation-Regulated Architecture of Injectable Smart Hydrogels Enhances Humoral Immune Response and Potentiates Antitumor Activity in Human Lung Carcinoma
8:25 am	**Christine Allen** University of Toronto	Harnessing Automation and Machine Learning for Sustainable Drug Formulation Development
8:45 am	**Alexander Kabanov** University of North Carolina	Morphology, Partitioning and Pharmacological Performance in Block Copolymer Systems
9.05 am	**Glen Kwon** University of Wisconsin	PEGylated Functional Upstream Domain Peptide from S. Pyogenes Disrupts Fibronectin Fibrillogenesis and Reduces Bleomycin-Induced Pulmonary Fibrosis
9:25 am	**Twan Lammers** RWTH Aachen University	Theranostic Strategies to Promote Polymeric Nanomedicine Clinical Translation
9:45 am	Coffee break	

Session 6 Moderator: Shawn Owen

Time	Speaker	Title
10:00 am	**You Han Bae** University of Utah	Mechanistic Pathway of Nanoparticle Ileal Absorption
10:20 am	**Yoon Yeo** Purdue University	Local Delivery of Paclitaxel and Nucleic Acids via an Immunoactive Polymer for Systemic Therapy of Solid Tumors
10:40 am	**Yue Lu** University of Utah	Digital Pharmaceutics: A Data-driven Approach for Precision Medicine
11:00 am	**Tejal Desai** University of California, San Francisco	Nanostructured Interfaces to Modulate Epithelial Transport
11:20 am	**Kidong Park** Ajou University	Injectable Hydrogels Releasing Reactive Oxygen/Nitrogen Species for Tissue Regeneration
11:40 am	**Patrick Stayton** University of Washington	Engineering Prodrug Therapies For Infectious Disease Therapy
12:00 pm	**David Grainger** University of Utah	Closing Remarks
12:15 pm	**Lunch**	

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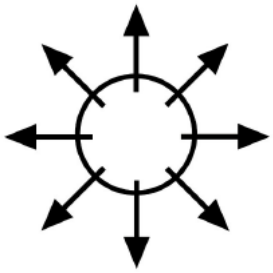

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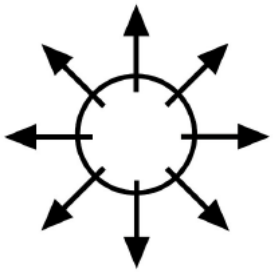
SPEAKER ABSTRACTS



James M. Anderson received his Ph.D. at Oregon State University in 1967, his M.D. degree from the Case Western Reserve University School of Medicine in 1976, and did his Anatomic Pathology residency at the Institute of Pathology of University Hospitals of Cleveland. Following the completion of his residency, he joined the faculty of the Institute of Pathology at Case Western Reserve University. Throughout his career James Anderson has received many honors and awards such as a NIH MERIT Award, the Elsevier Biomaterials Gold Medal Award, the Honoris Causa PhD Degree from the University of Geneva and the 2013 Acta Biomaterialia Gold Medal, amongst others. He is a founding member of the Society for Biomaterials, the Controlled Release Society, and AIMBE and serves as a consultant to the NIH, FDA, and ISO. He is an elected member of the National Academy of Medicine and the National Academy of Engineering. Dr. Anderson is the Chair of ISO 10993-Part 1, Biomedical Device and Biomaterial Biocompatibility. He was the Editor-in-Chief of the Journal of Biomedical Materials Research-Part A. Dr. Anderson has worked in the area of biomaterials, medical devices, and prostheses for the past 40 years and his activities ranged from the clinical pathology evaluation of retrieved implants from humans to fundamental studies of cellular interactions with biomaterials.

THE SUNG WAN KIM I KNEW

James M. Anderson

Departments of Pathology and Biomedical Engineering and Macromolecular Science, Case Western Reserve University, Cleveland, Ohio, 44106

Dr. Anderson will speak about the SUNG WAN KIM he knew and valued as a colleague, collaborator, mentor and friend.

Professor Jindřich Henry Kopeček is currently Distinguished Professor of Biomedical Engineering and Distinguished Professor of Pharmaceutical Chemistry at the University of Utah. He is an elected member of the US National Academy of Engineering (2011) and elected fellow of the US National Academy of Inventors (2018). Kopeček's research interests are focused on biorecognition of macromolecules, drug delivery systems, and self-assembled biomaterials. Hydrogels from his laboratory have been in clinical use and HPMA copolymer - anticancer drug conjugates in clinical trials. Kopeček's Hirsch index is 100; his publications have been cited 35,640 times (GoogleScholar 011422)

Smart Nanomedicines Based on Receptor Crosslinking

Jindřich Kopeček, Jiyuan Yang, M. Tommy Gambles, Jiahui Li, D. Christopher Radford

Center for Controlled Chemical Delivery, University of Utah, Salt Lake City, Utah 84112, USA

It is an honor to present at a symposium dedicated to the memory of Sung Wan Kim, a true pioneer in drug and gene delivery research. His contributions to the science of hydrogels, biodegradable drug conjugates, stimuli-sensitive polymers, and polymer-based gene-delivery systems were truly visionary. Sung Wan was an outstanding scientist but also a wise, modest man who enjoyed life, family, friends, and playing golf. He will live in our hearts; our lives have been shaped by his wisdom and legacy.

One of the research focuses in the Kopeček/Yang laboratory is receptor crosslinking; it can considerably enhance the rate of internalization and/or manipulate the subcellular fate of receptor-bound ligands. A recycling receptor, following crosslinking, may change its subcellular fate and carry the cargo to the lysosomal compartment.[1]

The lecture will present two examples of new nanomedicines where the receptor crosslinking was an important design principle:

Drug-free macromolecular therapeutics (DFMT) is a new paradigm for the treatment of B cell malignancies. Apoptosis is initiated by the biorecognition of complementary oligonucleotide motifs at the cell surface resulting in crosslinking of CD20 receptors. DFMT is composed from two nanoconjugates: a) bispecific engager, Fab'-MORF1 (anti-CD20 Fab' fragment conjugated with morpholino oligonucleotide 1), and b) a crosslinking (effector) component P-$(MORF2)_X$ or HSA-$(MORF2)_X$, (*N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer or human serum albumin grafted with multiple copies of complementary morpholino oligonucleotide 2).[2-5] Notably, the therapeutic efficacy of anti-CD20 mAbs (ADCC, CDC, and CD20-mediated apoptosis) requires immune effector cells to function. In contrast, DFMT triggers direct and specific apoptosis of B-cell malignancies without the help of effector cells. This is achieved by the design of synthetic effectors that reproduce the crosslinking function of immune effector cells.

The second generation of DMFT is based on Type II antibodies.[6] Anti-CD20 antibodies are divided into Type I such as rituximab (RTX) and Type II such as obinutuzumab (OBN); they have different patterns of binding to CD20 receptor resulting in different mechanism of action. RTX binds between CD20 tetramers resulting in accumulation in lipid rafts, calcium influx and caspase activation. OBN binds within one tetramer with the conformation compatible with homotypic adhesion regions, leading to actin cytoskeleton remodeling and lysosome disruption. Our design enhances the activity of Type II OBN by triggering the apoptosis activation pathways of both types of antibodies. This new system is composed of two nanoconjugates: a) bispecific engager, OBN-MORF1 (OBN conjugated to one morpholino oligonucleotide MORF1); and b) a crosslinking (effector) component HSA-$(MORF2)_X$ (human serum albumin (HSA) grafted with multiple copies of complementary morpholino oligonucleotide 2). Modification of OBN with one MORF1 does not impact the binding of OBN-MORF1 to CD20 and following binding to CD20 Type II effects occur. Further exposure to multivalent effector HSA-$(MORF2)_X$ results in clustering the OBN-MORF1-CD20 complexes into lipid rafts and Type I effects occur. This new approach, called "clustered OBN (cOBN)" combines effects of both antibody types resulting in high apoptotic levels.[5]

This principle has been applied to crosslinking of CD38 as well as simultaneous crosslinking of CD20 and CD38. The results suggest that these are promising approaches for the treatment of multiple myeloma.[4]

Combination chemo- and immunotherapy of breast cancer using backbone degradable HPMA copolymer – epirubicin conjugate (KT-1) with a multivalent polymer-anti-PD-L1 peptide antagonist (MPPA) is a polymer-assisted combination of immunogenic chemotherapy and PD-L1 degradation for efficacious treatment in originally non-immunogenic cancer. "Priming" tumors with backbone-degradable polymer-epirubicin conjugates elicits immunogenic cell death and fosters tumor-specific CD8+ T cell response. Sequential treatment with a multivalent polymer-peptide antagonist to PD-L1 overcomes adaptive PD-L1 enrichment following chemotherapy, biases the recycling of PD-L1 to lysosome degradation *via* surface receptor crosslinking, and produces prolonged elimination of PD-L1 rather than the transient blocking afforded by standard anti-PD-L1 antibodies. Together, these findings established the polymer-facilitated tumor targeting of immunogenic drugs and surface crosslinking of PD-L1 as a potential new therapeutic strategy to propagate a long-term antitumor immunity, which might broaden the application of immunotherapy to immunosuppressive cancers.[6,7]

Acknowledgement. The research was supported by NIH grant RO1 CA246716 from the National Cancer Institute (to JK) and Department of Defense grant W81XWH-20-1-0573 (to JY).

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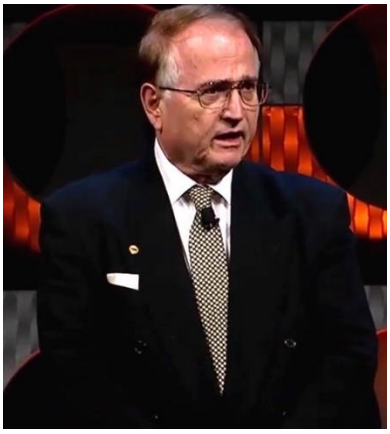

Nicholas A. Peppas is a professor in Biomedical Engineering, Chemical Engineering, Pediatrics, Surgery and Pharmacy at the University of Texas at Austin. His group has developed new drug delivery systems for oral, buccal, sublingual and gastrointestinal delivery of drugs, peptides and proteins. 1,400 publications, IF of H=192 (170,000 citations). Numerous US patents issued or pending, 3 start-up companies. Research in biomaterials, drug delivery, and bionanotechnology blends modern molecular and cellular biology with engineering principles to design next-generation medicines and devices. Awards include NAE Founders Award, NAM Adam Yarmolinsky Award, AAPS Global Leader and Distinguished Pharmaceutical Scientist Awards. Peppas is a member of sixteen Academies including NAE, NAM, American Academy of Arts and Sciences, National Academy of Inventors, Academia Europaea, International Academy of BME, Canadian Academy of Engineering, Indian National Academy of Engineering, Chinese Academy of Engineering, Korean Academy of Science and Technology, National Academy of France, Royal Academy of Spain, Academy of Athens, Greece, & Academy of Texas. He is the Editor-in-Chief of "Regenerative Biomaterials" (Oxford). He holds a Dipl. Eng. from NTU of Athens (1971), a Sc.D. from MIT (1973) and is the recipient of twelve honorary doctorates and professorships.

Cationic Nanogels for Oral Targeted siRNA Delivery to Macrophages for Treatment of Inflammatory Bowel Diseases

Nicholas A. Peppas, Olivia L. Lanier, Abielle P. D'Andrea, Phoebe C. Muller-McKinstry and Neha Krishnan

Departments of Biomedical and Chemical Engineering,
Departments of Pediatrics. Surgery and Perioperative Care, Dell Medical School
The University of Texas at Austin, Austin, TX 78712-1801

Introduction: RNA interference is an important treatment method that utilizes small interfering RNA (siRNA) to silence the production of specific strands of mRNA and thus reduce the production of that protein. It has been FDA approved for treatment of multiple diseases [1], and has promise as a treatment for inflammatory bowel diseases (IBDs) [2]. Current treatments for IBDs include immunosuppressant drugs and parenterally delivered biologics [3]. These treatments are delivered systemically and reduce immune system performance against normal pathogens and damage [4]. Further, parenteral delivery is not feasible for patients in poor areas of the world and requires meticulous care so as not to cause infection. Therefore, a *targeted* system that delivers siRNA *orally* to the site of inflammation in the intestines would be of immense benefit as it would reduce off-target immunosuppression and be more accessible to the global population. Several challenges associated with the oral delivery of siRNA include enzymatic degradation, extreme pH environments, targeting inflammatory cells, and the need to achieve intracellular delivery and endosomal escape while maintaining siRNA integrity [5]. We developed cationic nanogels to deliver siRNA to macrophages in the intestines after enteric delivery. Cationic nanogels were synthesized to be the appropriate size to undergo uptake by macrophages, and nontoxic with a pK_a to promote endosomal escape. The carriers can protect siRNA until after endosomal escape.

Methods: In this study cationic nanogels were synthesized using ARGET ATRP [6] with various cationic monomers: 2-(dimethylamino) ethyl methacrylate, 2-(diethylamino) ethyl methacrylate (DEAEMA) and 2-(diisoprpylamino) ethyl methacrylate. To adjust the pK_a to the pH of an endosome and to reduce toxicity, hydrophobic comonomers were added: tert-butyl methacrylate, cyclohexyl methacrylate, and hexyl methacrylate [7]. Additionally poly(ethylene glycol) methyl ether methacrylate was grafted to the nanogels to reduce aggregation and further reduce toxicity. Various formulations of nanogels were synthesized and characterized for swollen and collapsed size by dynamic light scattering, swelling ratio, pK_a by potentiometric titration, *in vitro* toxicity in multiple cell lines with MTS assay, and siRNA loading and release kinetics using QuantIT RNA detection assay. siRNA loading was performed for 1 hour (or 72 hours) at both charged (pH 5.5) and neutral (pH 7.5) conditions in buffer. Release was performed over 8 hours under sink conditions in buffers at both pH values.

Results: Increasing the hydrophobicity of the cationic monomer or increasing the content of hydrophobic comonomers in the particles increased the nanogel aggregation, reduced their volume swelling ratios, and reduced the pK_a. Therefore, it was determined that DEAEMA with a hydrophobic comonomer at 22 mole % should be used for desired pK_a and swelling behavior. Nanogels were nontoxic at concentrations of 50 nM in different cell models. Loading at pH 5.5 when nanogels were charged resulted in nearly 100% loading where loading at pH 7.5 resulted in less than 50% loading. siRNA was released within ~8 hours, although much of the encapsulated siRNA had been released within 4 hours. However, phagocytosis of polymeric particles by

macrophages has been shown to occur within minutes[8], so the quick release rate should be ideal for this application. To decrease the burst release, loading can be done under neutral pH conditions for longer time periods (72 hours). Release experiments were all done at pH 7.5, when nanogels are neutrally charged, because they have negligible release at pH 5.5 when charged.

Conclusions: Cationic nanogels that are nontoxic with appropriate swelling, size, and pKa were synthesized. siRNA loading and release was shown under multiple pH conditions to optimize the system for future experiments.

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Helen Cho, PhD received her Bachelor's degree in Biochemistry at Yonsei University and Master degree in Biological Engineering at Korea Advanced Institute of Science and Technology) in Korea. She moved to the US and studied how mRNA transcription was regulated by RNA polymerase II under the tutelage of Dr. Danny Reinberg at the University of Medicine and Dentistry of New Jersey. As a postdoctoral trainee she conducted research on transcriptional regulation mechanisms of coactivators and corepressors involved in nuclear hormone receptor signaling through epigenetic and posttranscriptional protein modifications at the Salk Institute for Biological Sciences with Dr. Ron Evans, Dr. Cho joined Global Pharmaceutical Company, Pfizer where she devoted her multi-disciplinary knowledge in developing vaccine based immunotherapy based regimens for the treatment of cancer. As a Program Leader, she led the research and development of cancer vaccine- and oncolytic virus-based immune-oncology combination therapies into early phase clinical trials. Recently, Dr. Cho returned to Korea to join Samyang Holdings Corporation as the director of R&D where she oversees the application research of proprietary biodegradable materials to surgical care and aesthetic products and nano-polymeric DDS technology into oncology products, including the research and development of Stability Enhanced NanoShell (SENS), a proprietary gene delivery platform.

Preclinical development of tissue-selective polymer-lipid based nanoparticle formulations for the delivery of mRNA vaccine and therapeutics

Helen Cho, PhD

Biopharmaceuticals Division, Samyang Holdings, Seoul, Republic of Korea

Samyang Holdings holds a rich history of polymeric nanoparticle-based formulation research, represented by Genexol®-PM and Nanoxel®-M as approved oncology products. Building upon the polymeric micelle (PM) drug delivery technology applied in Genexol®-PM and Nanoxel®-M products, combinations of proprietary polymers and cationic lipids that form stable nanoparticles with genetic materials and efficiently deliver the genetical material into cells of organs/tissues were characterized as SENS^{TM} platform technology. These SENS^{TM} formulations were optimized for the delivery of DNA, siRNA, and mRNA respectively. By controlling the compositions and mixing processes of SENS^{TM} formulations, selective tissue distribution of the genetic material to the spleen, lung, and liver etc. were achieved. We are currently applying the SENS^{TM} technology to mRNA therapeutics for the development of prophylactic and therapeutic cancer vaccines, protein replacement, and gene therapies. We continue to diversify the compositions and grow the SENS^{TM} library with the goal to enhance the therapeutic potential for a broader range of diseases with special delivery needs.

Shreya Goel is an assistant professor in the department of pharmaceutics and pharmaceutical chemistry at the University of Utah. Prior to that, she completed her postdoctoral fellowship from MD Anderson Cancer Center and her PhD degree from the University of Wisconsin-Madison. Her research program focuses on molecular imaging, nanomedicine and theranostics and she is widely published in these areas. In addition to her research and teaching activities, she currently serves as an associate editor for the Journal of Nanobiotechnology.

Multiscale Imaging in Nanomedicine: A Value Proposition

Shreya Goel

Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah, USA

Drug delivery approaches and systems have evolved tremendously in the past decades, owing to advances in physical sciences and engineering as applied to oncology. Many recent breakthroughs in experimental therapeutics have a multicomponent framework, formulated to act synergistically toward improving the delivery and efficacy of active drugs in a site-specific manner. Given the complexity in the properties of such systems, it is important and challenging to precisely monitor the in vivo transport and interactions within the body to determine the spatiotemporal variants of efficacy. Such information is conspicuously missing for drug delivery systems that target metastatic cancers. In this talk, taking the example of injectable nanoparticle generators encapsulated with polymeric doxorubicin (iNPG-pDox), I will describe how we can integrate high temporal resolution of in vivo whole-body PET-CT, ex vivo whole-organ optical imaging, and high spatial resolution of confocal microscopy, with mathematical modeling, to systematically deconstruct the trafficking of promising experimental therapeutics in metastatic breast cancer. This information can then be used to aid the design of improved drug delivery systems through a rational image-guided reverse-engineering approach. Overall, we demonstrate the utility of employing multiscale, multimodality imaging as a powerful toolbox that can complement current translational workflows to allow rapid and accurate evaluation of advanced complex therapies in preclinical and translational studies.

Jan Feijen is Emeritus Professor of Polymer Chemistry and Biomaterials at the University of Twente, Enschede, The Netherlands, Chair Professor at Soochow University Biomedical Polymers Laboratory, Suzhou, China and Adjunct Professor of Pharmaceutics at the University of Utah, Salt Lake City, USA. From 1995 – 2009 he has been the Scientific Director and Founder of the Institute for Biomedical Engineering, BMTI, at the University of Twente. His research interests are biomaterials, biodegradable polymers, bio-interfacial phenomena, ring-opening polymerization, hydrogels, drug delivery systems and nano-systems for cancer therapy. He trained 90 PhD students and has (co)-authored over 600 papers with more than 39.000 citations, H-index 102 (Web of Science), H-index 104 (Scopus) and H-index 123 (Google Scholar) and holds 38 patents. He has served on many editorial boards, was the co-founding editor of the J. Controlled Release and has been guest editor for the Journal for many years. Since 1990 he has organized and was the chairman of the first 10 European Symposia on Controlled Drug Delivery Systems, The Netherlands and this series of biannual Symposia is still continuing. Since 2010 he has been the co-organizer of the biannual Symposia on Innovative Polymers for Controlled Delivery (SIPCD) in Suzhou, China. In 2005 he initiated and became a chairman of the Dutch program for Tissue Engineering (DPTE) with a budget of 50 million Euro's. He is a Fellow of the American Society for Biomaterials, since the start, and a Fellow of the Controlled Release Society. He was former governor of the Controlled Release Society, European Society for Biomaterials and International Society for Artificial Internal Organs. He was a chairman for five years of the Concerted Action on Heart and Replacement Technology, Medical Health Research Programme of the European Community. He has received numerous prestigious awards including the Best Paper Award of the J. Controlled Release, Controlled Release Society (1992), the Clemson Award, American Society for Biomaterials (1994), the Founders Award, Controlled Release Society, (1998), the George Winter Award, ESB, (2002) and the Award for Distinguished Service in Advancement of Biomaterials Science, Japanese Society for Biomaterials, (2008). He also received the Honorary Medal of the University of Twente (2008) and in 2003 he received the Royal Decoration, Knight of the Order of the Netherlands Lion. In 2018 he became an Honorary Member of the European Society of Biomaterials. He was a highly cited author in the field of Pharmacology and Toxicology in 2018 and 2019 (Publon).

In Memory of Prof. Dr. Sung Wan Kim

Jan Feijen

University of Twente, Enschede, The Netherlands

Dear Friends, Colleagues and Family,

February 24th , it has already been two years ago that Sung Wan passed away. Due to the Covid situation, I was not able to be present at his funeral.

In 1972, I met Sung Wan and his family for the first time in Salt Lake City. Since that time we became friends and worked closely together in the fields of biomaterials and drug delivery.

In this short presentation I will share with you my personal memories about his scientific achievements, our scientific cooperation and our lifelong friendship.

[1] J. Feijen, In memory of Prof. Sung Wan Kim , Obituary, J. Control. Release, 321 (2020), pp. 773-774
[2] J. Feijen, The triangle, in memory of Prof. Sung Wan Kim , J. Control. Release, 328 (2020), pp. 962-969

Wim E. Hennink Professor and head of the department of Pharmaceutical Sciences, Utrecht University Wim Hennink obtained his Ph.D. degree in 1985 at the Twente University of Technology on a thesis with a biomaterials research topic. From 1985 until 1992 he had different positions in the industry. In 1992 he was appointed as professor at the Faculty of Pharmacy of the University of Utrecht. From 1996 on he is head of the Pharmaceutics division. At present he is the head of the Department of Pharmaceutical Sciences, Utrecht University. His main research interests are in the field of polymeric drug delivery systems. He published over 600 papers and book chapters and is the inventor of 20 patents.

Stability of drug-loaded polymeric micelles

Wim E. Hennink, Masha Bagheri, Aida Varela-Moreira, Yan Wang, Yang Shi

Department of Pharmaceutics, Utrecht University, the Netherlands

Most amphiphilic block copolymers spontaneously form polymeric micelles in aqueous solutions above the critical micelle concentration. Generally speaking, polymeric micelles are characterized by a core-shell structure with sizes that range from 10-100 nm. The hydrophilic shell ensures their colloidal stability whereas the hydrophobic core can be used for the loading of particularly hydrophobic drugs. Because of these attractive features, polymeric micelles are under investigation as drug delivery system and some formulations have reached clinical evaluations [1,2]. It should be noticed, however, that polymeric micelles are dynamic systems which means that destabilization might occur when their concentration drops below the CMC. Further, the loaded drugs can be rapidly released in biological media due to the presence of proteins that act as solubilizers of the drugs. (e.g., albumin, lipoproteins). To enhance the stability of polymeric micelles, strategies to chemically crosslink polymeric micelles and also to covalently link the drug to the core have been exploited. To ensure biodegradability, crosslinking/coupling methods are used in which bonds are present that degrade under physiological conditions (e.g., ester and disulfide bonds) [3, 4]. To avoid the use of the chemical methods, physical strategies have also been investigated to increase the stability of polymeric micelles [5]. In our research programme, we have employed π–π stacking to increase the stability of polymeric micelles as well as the retention of loaded drugs using block copolymers of PEG and pHPMA-Bz (poly(*N*-2-benzoyloxypropyl methacrylamide). It was shown that the anticancer drugs (paclitaxel and docetaxel) were well retained in the micelles, even in the circulation, and good therapeutic efficacy was seen in different animal models [6, 7]. In another study, we solubilized the pharmacological active compound curcumin in the same polymeric micelles. Although the micelles showed good stability in the circulation, the loaded curcumin was rapidly extracted from the micelles [8]. Although curcumin, just as paclitaxel and docetaxel, is a compound with a high log P and also contains aromatic groups, the reasons for its fast release in blood are not fully understood yet. Recently we developed polymeric micelles composed of amphiphilic block copolymers of pHPMA and pHPMA-Bz with and without a biotin terminus [9,10]. These biotinylated micelles loaded with different hydrophobic drugs were incubated in buffer, plasma and even full blood to study their release characteristics. Due to the biotin decoration, these micelles could be removed from the release medium using streptavidin-coated magnetic beads, allowing quantification of both the released drug and the amount of drug still retained in the micelles. This method confirms our previous findings that paclitaxel was better retained in the micelles than curcumin.

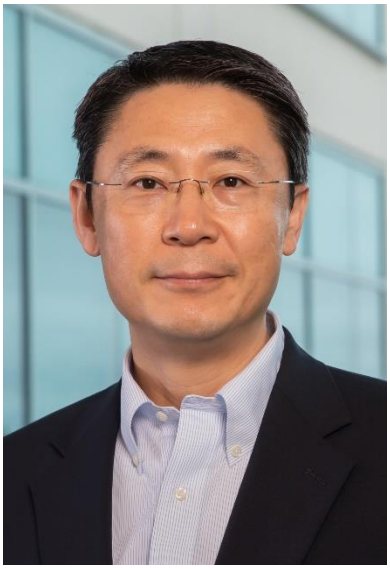
Suzie H. Pun is the Washington Research Foundation Professor of Bioengineering, an Adjunct Professor of Chemical Engineering, and a member of the Molecular Engineering and Sciences Institute at UW. She is a fellow of the U.S. National Academy of Inventors (NAI) and American Institute of Medical and Biological Engineering (AIMBE), and was been recognized with MIT Technology Review's "Top 100 Young Innovators" designation, the Presidential Early Career Award for Scientists and Engineers, the Young Investigator Award from the Controlled Release Society, and as an AAAS-Lemelson Invention Ambassador. She was also recognized with the University of Washington's Marsha Landolt Distinguished Graduate Mentor Award for her dedicated mentoring of students. She currently serves as an Associate Editor for ACS Biomaterials Science and Engineering. Suzie Pun received her B.S. in Chemical Engineering from Stanford University and her Ph.D. in Chemical Engineering from the California Institute of Technology. She also worked as a senior scientist at Insert Therapeutics/Calando Pharmaceuticals developing polymeric drug delivery systems before joining the Department of Bioengineering at University of Washington. Her current work focuses on biomaterial applications in drug delivery and gene and cell therapy.

VIPER: A Virus-Inspired Polymer for Endosomal Release

Suzie H Pun

Professor of Bioengineering, University of Washington
Seattle, Washington, United States of America

Drug carriers that mediate intracellular delivery of macromolecules remains an important technology for realizing clinical translation of many nucleic acid, peptide and protein drugs. We have developed a pH-sensitive block copolymer, called VIPER (for “Virus-Inspired Polymer for Endosomal Release”), that facilitates intracellular delivery of nucleic acids and peptides based on design principles from adenovirus. In this presentation, I will discuss briefly the design and development of VIPER and share our results using VIPER for gene delivery, cytotoxic peptide delivery and peptide antigen delivery.



Jinming Gao is a Professor of Oncology, Pharmacology, Otolaryngology and Cell Biology at UT Southwestern Medical Center. He holds the Elaine Dewey Sammons Distinguished Chair in Cancer Research, in Honor of Eugene P. Frenkel, M.D. Dr. Gao is a scientific founder and chief scientific officer of OncoNano Medicine, a biotech startup spun out of UT Southwestern. His lab invented the ultra pH-sensitive nanoparticle technology that constitutes the pharmaceutical pipeline at OncoNano Medicine. Dr. Gao has served as the past chair of the Gene and Drug Delivery study section and is a member of the Advisory Council to the Center for Scientific Review at the National Institutes of Health. Dr. Gao is currently leading a Beau Biden Cancer Moonshot U54 Nano-Immune-Engineering Center to integrate molecular immunology, biomedical engineering and clinical oncology to advance cancer immunotherapy.

Nano-Immuno-Oncology: Harnessing Molecular Cooperativity for Cancer Immunotherapy

Jinming Gao

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Abstract

Nano-immuno-oncology is an emerging cross discipline that harnesses nanotechnology's unique synergy with immunology to advance cancer immunotherapy. Human immune system has evolved to sense and respond to nano- and micro-particulates (e.g., viruses, bacteria). Through the versatile control of composition, size, shape, and surface properties of nanoparticles, nano-immune-engineering approaches are uniquely positioned to mount appropriate immune responses against cancer. In this presentation, I will discuss how molecular cooperativity principles can be designed to create multi-component nanosystems with emergent physical and biological properties where the whole is bigger than the sum of its parts. Such examples include ultra-pH sensitive nanoparticles that target deregulated tumor energetics for tumor-activatable delivery of imaging or therapeutic payloads, or non-canonical STING activation with prolonged type I interferon responses to augment antitumor immunity.

Antonios G. Mikos Rice University Antonios G. Mikos is the Louis Calder Professor of Bioengineering and Chemical and Biomolecular Engineering at Rice University. His research focuses on the synthesis, processing, and evaluation of new biomaterials for use as scaffolds for tissue engineering, as carriers for controlled drug delivery, as non-viral vectors for gene therapy, and as platforms for disease modeling. His work has led to the development of novel orthopaedic, dental, cardiovascular, neurologic, and ophthalmologic biomaterials. He is the author of over 670 publications and the inventor of 32 patents. Mikos is a Member of the National Academy of Engineering, the National Academy of Medicine, the National Academy of Inventors, the Chinese Academy of Engineering, the Academia Europaea, and the Academy of Athens. He has been recognized by various awards including the Lifetime Achievement Award of the Tissue Engineering and Regenerative Medicine International Society-Americas, the Founders Award of the Society For Biomaterials, the Founders Award of the Controlled Release Society, the Acta Biomaterialia Gold Medal, and the Robert A. Pritzker Distinguished Lecturer Award of the Biomedical Engineering Society. He is a founding editor and editor-in-chief of the journal Tissue Engineering

Injectable and 3D-Printable PNiPAAm-Based Hydrogels for Tissue Engineering

Emily Y. Jiang, Adam M. Navara, Hannah A. Pearce, and Antonios G. Mikos

Rice University, Houston, USA

Thermoresponsive polymers are a powerful tool in biomaterials and tissue engineering. Due to their sensitivity to temperature change, these polymers have been leveraged for injectable, 3D printing, and drug delivery applications as the polymers can be designed to undergo sol to gel transition at specific temperatures[1–5]. One of the most significant contributors to this field is the late Professor Sung Wan Kim. Prof. Kim's impactful work investigating thermoresponsive polymers and other stimuli-responsive polymers as a drug delivery platform has shaped the field of pharmaceutics[6–9]. During his productive career, Prof. Kim published more than 800 papers utilizing polymers such as poly(ethyleneimine), poly(amido amine), and thermally responsive caprolactone-, poly(ethylene oxide)-, and poly(ethylene glycol)-based block copolymers for the delivery of genes, drugs, and RNA *in vitro* and *in vivo*[10–14]. Building from his pioneering work, thermoresponsive polymers are an active area of research within biomaterials and tissue engineering. One of the most notable polymers in the field is poly(*N*-isopropylacrylamide) (PNiPAAm), in part due to its ability to copolymerize with other acrylate-bearing monomers. Additionally, PNiPAAm has a lower critical solution temperature (LCST) of ~32 °C, creating a polymer solution that will gel when placed in a physiologic environment[3].

Recent efforts in our laboratory have been focused on injectable and 3D printable PNiPAAm-based hydrogels for osteochondral tissue engineering. Though thermal gelation ensures a sol to gel transition of the polymer, for many biomaterials applications, a crosslinked network is desirable to permit the infiltration of cells and perfusion of drugs and other factors. A novel functionalizable crosslinker, poly(glycolic acid)-poly(ethylene glycol)-poly(glycolic acid)-di(but-2-yne-1,4 dithiol) (PdBT), was developed in our laboratory[1]. PdBT crosslinks a PNiPAAm-co-glycidyl methacrylate-based macromer via thiol-epoxy chemistry and can be modified with bioactive molecules via alkyne-azide chemistry. PNiPAAm-based hydrogels crosslinked with PdBT have been shown to possess robust physicochemical properties suggesting an open, crosslinked network[1,15]. The functionality of the PdBT crosslinker in allowing the covalent tethering of bioactive factors has enabled PNiPAAm-based gels to promote chondro- and osteo-induction *in vitro* and *in vivo*[16,17].

A PNiPAAm-based copolymer has also been successfully adapted as an ink in 3D bioprinting. Our laboratory has developed a four-part thermogelling macromer consisting primarily of PNiPAAm that can be 3D printed and then crosslinked with a poly(amidoamine) crosslinker to form multilayered scaffolds[3]. The thermogelling behavior of the PNiPAAm allows the ink to be extruded as a liquid below its LCST, into a thermogelling poloxamer support bath heated to 37 °C, where it then immediately thermally gels into a defined polymeric fiber. Leveraging the thermogelling behavior of the ink in this way allows smaller nozzles to be used, as the PNiPAAm ink has a much lower viscosity in its liquid state, thus increasing the overall resolution attainable by the system to fiber diameters between 80 and 200 μm. This system was used to print viable encapsulated fibroblasts, demonstrating the potential for this PNiPAAm-based ink to be utilized in future high-resolution bioprinting applications.

Thermogelling polymers like PNiPAAm have been a groundbreaking material for work in our laboratory and many others. These advances have demonstrated considerable potential in improving health outcomes – advances that were made possible by the pioneering work Professor Kim conducted during his illustrious career.

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Ram I. Mahato is a Professor and Chairman of the Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE. He was a professor at the University of Tennessee Health Science Center, Research Assistant Professor at the University of Utah (with Sung Wan Kim), Senior Scientist at GeneMedicine, Inc., and as a postdoctoral fellow at the University of Southern California in Los Angeles (with Vincent HL Lee), Washington University in St. Louis, and Kyoto University, Japan (with Mitsuru Hashida). He received PhD in Drug Delivery from the University of Strathclyde, Great Britain and BS from China Pharmaceutical University, Nanjing. He has published 166 peer reviewed papers, 24 book chapters, holds 3 US patents, and has edited/written nine books and eleven journal issues (Total Google Citations= 13,020 and h-Index =68). He is an Associate Editor of the Journal of Neuroimmune Pharmacology, was a Feature Editor of the Pharmaceutical Research (2006-2013) and is the Editorial Board Member of eight journals. He is a CRS Fellow (2011), AAPS Fellow (2010), Permanent Member of BTSS/NIH Study section (2009-2013), and ASGCT Scientific Advisor (nonviral vectors, 2006-2009). He is applying sound principles in pharmaceutical sciences in the context of the latest advances in life and material sciences to solve challenging drug delivery problems in therapeutics. His areas of research include delivery and targeting of small molecules, miRNA and genes using novel polymeric and lipid carriers for treating cancer, liver fibrosis and diabetes.

Polymeric Nanomedicine of Multiple Small Molecule Drugs for Treating Medulloblastoma

Ram I. Mahato

Department of Pharmaceutical Sciences, University of Nebraska Medical Center, USAs

Treatment of medulloblastoma (MB) is challenging due to diverse genetic make-up, resistance to chemotherapy, inefficient drug transport across the blood brain barrier (BBB) and drug induced neurotoxicity. Hedgehog (Hh) and IGF/PI3K signaling pathways regulate cell growth, cancer stem cell (CSC) proliferation, and tumorigenicity in MB patients. Hh inhibitors are effective initially to treat SHH-MB, but their repeated use develops chemoresistance but can be overcome by modulating Gli, which is downstream of Smo using SF2523, which is a BRD4/PI3K dual inhibitor and inhibits MYCN expression. We synthesize Smo inhibitor 2-chloro-N1-[4-chloro-3-(2-pyridinyl) phenyl]-N4, N4-bis (2-pyridinyl methyl)-1, 4-benzene-dicarboxamide (MDB5). MDB5 and SF2523 effectively inhibited the proliferation of ONS-76 and HD-MB03 cells, with significantly higher cell killing when these drugs were used in combination. Treatment of HD-MB03 cells with the combination of these two drugs showed significantly higher decrease in colony formation and cyclin D1 expression, compared to individual drugs. We synthesized mPEG-b-PCC-g-DC copolymer, with 5.1 and 6.5% loading for MDB5 and SF2523 when formulated into nanoparticles (NPs). There was sustained drug release from NPs, wherein 100% of MDB5 was released in 50 h, but only 60% of SF2523 was released in 80 h. Targeted NPs were prepared by mixing COG-133-PEG-b-PCC-g-DC and mPEG-b-PCC-g-DC at 10:90, 20:80 and 30:70 ratios, with the highest cellular uptake at 30:70 ratio. Systemic administration of COG-133-NPs loaded SF2523 and MDB5 into orthotopic SHH-MB tumor bearing NSG mice resulted in significantly higher distribution to the brain at 6 and 24h post administration compared to non-targeted NPs loaded with these drugs while systemic injection of free drugs showed negligible drug concentrations in the brain. Moreover, systemic administration of COG-133-NPs loaded with MDB5 and SF2523 resulted in decreased tumor burden compared to non-targeted NPs of MDB5 and SF2523, with no hepatic toxicity. Successful completion of this project will provide a platform technology for treating SHH-MB and other brain tumors using this innovative NP-based combination therapy of Hh and BRD4/PI3K inhibitors.

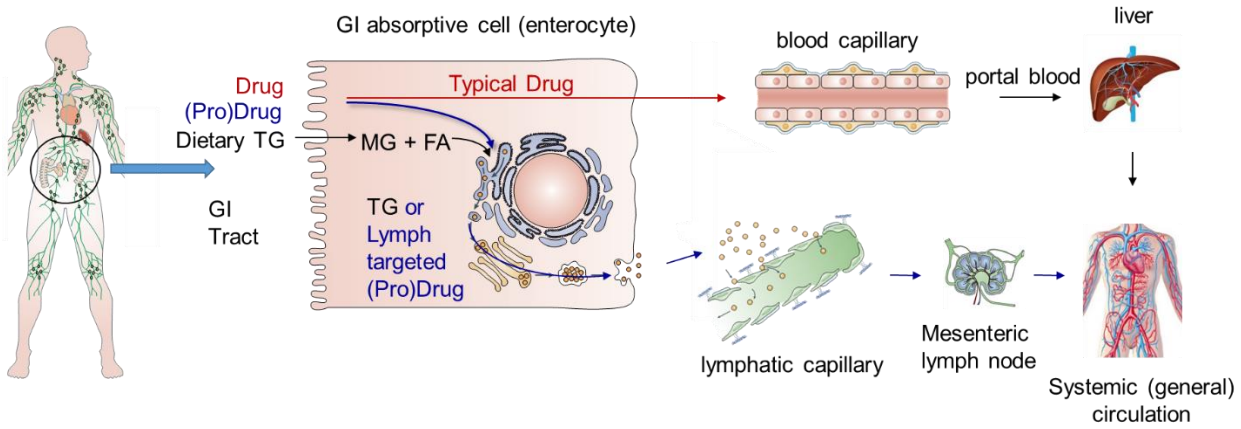
Professor Chris Porter is Director of the Monash Institute of Pharmaceutical Sciences (MIPS) at Monash University. Chris completed a Pharmacy degree and PhD in drug delivery at the University of Nottingham in the UK before moving to Australia to pursue an academic career. His personal research programs have subsequently focused on understanding and quantifying drug absorption, distribution and elimination profiles and on developing novel formulation approaches to optimize these properties. Major interests include improving the absorption of poorly water-soluble drugs, the role of the lymphatic system in drug absorption and the potential utility of dendrimers and other nanomaterials as drug delivery systems. Chris has published more than 250 peer reviewed papers (>23,000 citations, h-index 80, HiCi 2015, 2016 and 2018) and his research programs have attracted >$35m in funding. He is an inventor on >20 separate patent families, many of which are the subject of licensing/assignment deals. The most significant of these are with Starpharma (Melbourne) to develop the DEP® dendrimer-based targeted delivery system (currently in Phase 2 clinical trial) and with PureTech Health (Boston) to develop the Glyph®, lymphatic targeting technology (Phase 1 clinical trial).

Triglyceride-mimetic prodrugs to enhance lymphatic transport

Christopher Porter

Monash Institute of Pharmaceutical Sciences, Melbourne, Australia

After oral administration and absorption across the enterocyte, the majority of nutrients and drugs are taken up into the blood capillaries that drain the mesentery and from there trafficked via the hepatic portal vein, through the liver to the systemic circulation. In contrast, dietary triglycerides are digested in the intestinal lumen, absorbed and then trafficked to the enterocyte endoplasmic reticulum where they are re-esterified and enter into lipoprotein assembly pathways. This results in the generation of intestinal lipoproteins, mostly chylomicrons, that are taken up into lymphatic capillaries rather than blood capillaries. Specificity for lymphatic uptake occurs because chylomicrons are large and blood capillaries have tight junctions between endothelial cells that are relatively impermeable. In contrast, lymphatic capillaries have open button-like junctions between endothelial cells and are more permeable, allowing chylomicrons ingress.



Targeting drugs to this intestinal lymphatic transport pathway has two major advantages. Firstly, drugs in lymph are delivered at high concentration to the intestinal (mesenteric) lymph node. This is the site of immune response to gut-based antigens such as from food, microbiota or self-antigens (in gastrointestinal autoimmune diseases). Enhanced delivery to the mesenteric lymph node can thus enhance immunomodulation. Secondly, the lymphatic vessels drain from the mesenteric lymph node to the thoracic lymph duct and from there empty into the systemic circulation via the major veins in the neck. This provides an uninterrupted pathway to the rest of the body, avoiding first pass metabolism inherent in absorption via the blood.

In light of these advantages, a focus of my laboratory over the past several years has been the design and development of prodrugs based on drug conjugation to a chemical backbone based on dietary lipids. These conjugates can ‘piggyback’ onto the lipid absorption/lymphatic transport pathways in the gut, delivering them specifically and in high concentration to the lymphatics that drain the small intestine.

This presentation will discuss our recent work in this area with our partner PureTech Health including the development of an oral prodrug of natural allopregnanolone that has recently entered Phase 1 clinical trial.

Byeongmoon Jeong received his B.S. (1987) in the Department of Chemistry from Seoul National University, M.S (1989) from KAIST, and his Ph.D. (1999) in the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. He worked at LG Chem (1989-1994) and the Pacific Northwest National Laboratory, USA (1999-2002) as a senior research scientist before joining Ewha Womans University in 2002. He has authored 145 international peer-reviewed papers and patents on stimuli-sensitive polymers, of which 80 papers and 10 patents are related to biodegradable thermogels. His publications have been cited 10,800 times. He received an Achievement Award in the Polymer Division of Korean Chemical Society (2010) and Hanwha Polymer Award (2021) in Polymer Society of Korea. He was selected as Fellow of Biomaterials Science and Engineering (FBSE) nominated by International Union of Societies for Biomaterials Science and Engineering at World Biomaterials Congress (2020). He is serving as vice president of Korean Society of Biomaterials (KSBM, 2017~present) and Editor in Chief of the Biomaterials Research (2019~present), an official journal of KSBM. His research concentrates on stimuli-sensitive hydrogels and their applications for drug delivery and cell & tissue engineering.

Biodegradable Thermogels for 3D Cell Culture

Byeongmoon Jeong

Department of Chemistry and Nanoscience, Ewha Womans University, Seoul, Korea

Biodegradable thermogels have been extensively investigated for last decades. Due to the simple procedure for incorporating cells or bioactive agents, thermogels can be a promising materials for drug delivery and tissue engineering applications. Stating from poly(lactide/glycolide)-based thermogel, a series of thermogels including polycaprolactones, poly(trimethylene carbonate), disulfide- and polypeptide-based thermogels have been developed. In particular, polypeptide thermogels exhibited stability under in vitro conditions, and degradation occur under in vivo conditions. pH was maintained during degradation. Such properties enable the polypeptide thermogels to be promising carriers for biopharmaceuticals s including proteins, cells, and stem cells. In this presentation, we will summarize the development of thermogelling materials from historical point of view and introduce the recent biomedical applications of the thermogels focusing on stem cell research.

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Professor Yong-Hee Kim had a very intimate academic and personal relationship for 34 years before he passed away as a disciple of the late Professor Sung Wan Kim. After receiving Ph.D. under the supervision of Professor William I. Higuchi, he joined the late Professor Sung Wan Kim's lab as a Postdoctoral Fellow (1992-1994) in the Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, USA. Currently, he is a Professor and HYU Distinguished Research Fellow at the Department of Bioengineering, and Director of the Institute for Bioengineering and Biopharmaceutical Research, Hanyang University, Korea. He is a Distinguished Scientist (Einstein Professorship) for Chinese Academy of Sciences (CAS) President's International Fellowship Initiative, China. His research interests are focused on developing Novel Biopharmaceutical Delivery Platforms including Non-viral Gene Delivery (plasmid DNA, siRNA, mRNA), Targetable Nano- and Micro-particular Systems, Biomembrane-inspired Functional Nano-particular Systems, Immunomodulatory Combination Therapy, Transdermal Micro-array Systems, Targeted Oral Gene Delivery, Fusion Protein Delivery, and Injectable In-situ Gel Depot System. His scientific activities are Co-chair of the 2024 World Biomaterials Congress Organizing Committee, Fellow of the International Union of Societies for Biomaterials Science and Engineering, Former President of the Korean Society for Biomaterials, and Editorial Board Members of the Biomaterials and Archives of Pharmacal Research.

The Late Professor Sung Wan Kim-inspired Oligopeptide Complex for Targeted Non-viral Gene Delivery

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Gene therapy is the therapeutic approach of introducing foreign genomic materials into host cells to elicit therapeutic benefits by replacing defective genes or enhancing protein expression with DNA, or by reducing protein expression with siRNA, miRNA, or shRNA. The recent outbreak of the COVID-19 pandemic has drawn international attention to the potential of gene delivery systems. Advanced genetic engineering and nanotechnology have led to the rapid emergence of mRNA vaccines for unexpected infectious diseases. Although people all over the world have inoculated the lipid nanoparticle-based genetically-engineered mRNA vaccines, storage, maintenance, and effectiveness issues caused by the unstable structure of mRNA and the limitations of gene carriers still need to be improved. Accordingly, global pharmaceutical companies and researchers are focused on developing various gene delivery technologies. Undoubtedly, the impact of gene delivery systems in the pharmaceutical industry will be enormous in the future.

Nona-arginine (9R), one of the most effective protein transduction domains (PTDs), has shown high transduction ability for various cargoes, including DNA and siRNA. We have reported effectiveness of a reducible gene carrier, poly(oligo-D-arginine) (rPOA) based on 9R, by introducing internal disulfide bonds via spontaneous oxidation of the cysteinyl sulfhydryl groups, which demonstrated cytoplasm-sensitive reduction and efficient delivery of plasmid DNA and siRNA in various disease models. The reducible oligo-peptoplex was proven to be a promising tool for DNA or siRNA delivery in terms of improved stability in extracellular spaces, rapid dissociation in intracellular environments, minimum toxicity, stable transfection, and silencing activities *in vivo*.

For disease cell-targeted gene delivery, targeting moieties were combined with 9R/gene complex. Adipose tissue targeting oligopeptide (ATS9R, CKGGRAKD9RC) has been reported in our previous studies for selective adipose tissue-targeted delivery of diverse gene platforms in obesity and obesity-induced diabetes models. ATS9R was shown to have a specific binding affinity

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Nanomaterials for modulation of tumor immune microenvironments

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Nanostructures such as nanosheets, and nanoballs have been studied for delivery of chemical anticancer drugs and oligonucleotides. Although numerous studies have been done, the translation to commercialized products has not been successful. We aimed to design nanosystems which can modulate the immune microenvironment of tumors. For immunotherapy, adjuvant-loaded nanoparticles were modified with immune checkpoint blockade. In tumor-bearing xenograft, the surface-modified nanostructures provided selective activation by cleavage of fibroblast-associated protein at tumor microenvironment, and greater antitumor effect than other comparison groups. Moreover, we designed an adjuvant-entrapped nanoparticle which can assemble with tumor antigens in situ for effective activation of immune cells. The systemic administration of adjuvant-entrapped nanoparticles with near infrared light irradiation increased the activity of immune cell infiltration to the tumor cells, and inhibited tumor growth. In another study, we used gene-editing technique for sustained release of PD-1 aptamer from rolling circle-amplified DNA polyaptamer hydrogel. The Cas9/sgRNA-mediated release of the PD-1 DNA aptamer from the hydrogel activated the cytokine secretion of splenocytes, and increased the infiltration of immune cells to the tumor microenvironments. In another study, we used gene-editing technique for restructuring of tumor immune microenvironment. The delivery of plasmid DNA encoding Cas9 and TGF- specific sgRNA using lipid nanoparticles increased the infiltration of immune cells to the tumor microenvironments. Taken together, these studies provide potentials of modulating immune microenvironment of tumors for potentiated immunotherapy.

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Multifunctional Envelope-type Nano Device from Controlled Intracellular Trafficking to Clinical Application for Nanomedicines

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After Pandemic of COVID-19 in 2020, activities in human life were limited drastically and we waited for an efficient vaccine to be developed. Although it took ten years for a new vaccine to be developed usually, a new mRNA vaccine was developed and introduced into clinics within one year. The FDA approved mRNA vaccine developed by Pfizer/BioNTech in December 11th 2020, which is within one year since genome sequence of COVID-19 was shown in January 15th of 2020. It was unbelievable that a novel mRNA vaccine based on a cutting edge technology has been developed and approved for clinical usage within one year. A fantastic collaboration to achieve this miracle event will be discussed [1] and the latest progress of our own research related to this breakthrough will also be discussed.

We have been developing multifunctional envelope-type nano device (MEND) which can control not only in vivo biodistribution of drug carriers as well as cargos (nucleic acids, genes, proteins, etc.) but also intracellular trafficking of these materials (endocytosis for cellular entry mechanism, endosomal escape, translation/silencing of genes, organelle targeting such as nuclear entry, mitochondrial entry, etc.)[2]. To optimize these processes and achieve the maximum efficiency, we have examined many kinds of strategies and recognized that an ionizable cationic lipid is one of the most efficient strategy among those we experienced [3]. The first successful lipid was YSK05, which can induce gene silencing in liver hepatocytes of ED50 at 0.06 mg of siRNA/kg, which is 17-fold more efficient than original-MEND (1 mg/kg). The lipid nanoparticles (LNP) based on YSK05 encapsulating specific siRNA which are developed by Dr. Kohara were applied successfully to cure HCV infected chimeric mice [4]. A newly designed YSK13-LNP can cure HBV infected chimeric mice which are more difficult to treat than HCV [5].

To overcome the efficiency of MC3, which has become a gold standard for an ionizable cationic lipid and has been used in Onpattro®, the first siRNA nanomedicine approved by FDA in 2018, we have developed a library of ionizable cationic lipids by modifying structures not only hydrophilic head groups but also hydrophobic lipid tails [6]. One of the most efficient lipid was CL4H6, which can induce gene silencing in liver of ED50 at 0.0025 mg/kg, which is more efficient than MC3 (0.005 mg/kg). Successful examples using CL4H6 will be introduced not only for siRNA/mRNA delivery but also for genome editing and cancer immunotherapy [7].

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My name is **Youngro Byun**, a professor in College of Pharmacy in the Seoul National University. I received a Ph.D. in 1994 from the University of Utah. My supervisor then was Professor Sung Wan Kim. I worked at the University of Michigan as a Post-Doc fellow (1994-1996). My research field includes Targeting Anticancer Drug Delivery and Oral Drug Delivery. For Targeting Anticancer Drug Delivery, I have focused on targeting mKRAS and/or PTEN loss cancers. Also, I have developed the new cancer targeting molecule such as Doppel, and ADC by using Doppel mAb. For Oral Drug Delivery, I have focused on peptide drugs such as liraglutide and anticancer drugs. I have published over 250 papers and about 50 patents. I have worked as handling editor of Biomaterials.

The strategy to treat pan-KRAS mutant cancers by using albumin binding caspase-3 cleavable peptide-drug conjugate

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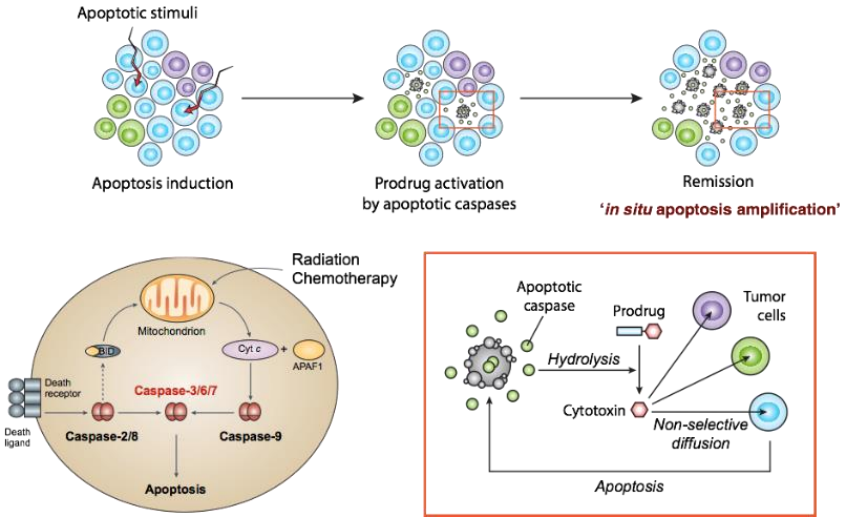
Despite current progress on developing KRAS direct targeted anti-cancer agents, yet there is no clinically available therapy for treatment of pan-KRAS mutant cancers with different KRAS gene mutations. In this study, a novel strategy for efficiently targeting pan-KRAS mutant cancers using a peptide-drug conjugate (PDC) was proposed. Also, existence of the genomically and epigenomically diverse subclones in a tumor severely limits the therapeutic efficacy of targeted agents. To overcome such a limitation, we prepared a novel targeted prodrug. EMC-DEVD-s-DOX, which comprises two important features: radiation-induced apoptosis targeting and albumin-binding properties. In particular, the prodrug binds circulating albumin after intravenous administration and then activated by caspase-3, which is upregulated from apoptotic cells that responded to radiotherapy. The prodrug was designed to bind circulating albumin to extend half-life and facilitate tumor accumulation in order to increase the possibility of contacting caspase-3, which is only transiently upregulated during apoptosis. Our results showed that EMC-DEVD-s-DOX had a prolonged half-life with enhanced tumor accumulation, which clearly benefited the therapeutic effect of the prodrug. Also, agreeing with the in vitro studies that showed ignorable cytotoxic effect in the absence of caspase-3, the prodrug was effective only when combined with radiotherapy without any noticeable systemic toxicity in vivo. Due to the highly selective action of EMC-DEVD-s-DOX independent to the complex genomic profiles of tumor, the prodrug would overcome the limitation of current targeted therapy and potentiate radiotherapy in the clinical oncology.



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Juvenile Chondrocyte Sheet-based Allogeneic Cartilage Regenerative Therapy

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Laboratory demonstrations of tissue engineering and cell therapy concepts have rapidly advanced with myriad cell sources and target disease models. However, unreliable, inconsistent human efficacy and limited therapeutic product scalability are critical barriers to bringing these promising new cell therapy technologies to the public at practical levels.

Cell sheet technology using temperature responsive cultureware allows the production of confluently cultured cells as sheets. Harvested sheets are readily patched on tissue lesions and adhere without suture, fibrin glue or other adhesion techniques. Several types of autologous cell sheets have been successfully delivered to small numbers of patients to date with clinical safety and efficacy in seven organs[1–7].

Cartilage lacks an innate repair capability after traumatic injury and a gold standard regenerative treatment algorithm has not been established. Cell sheets are a unique approach that grafts a highly cell-dense patch directly to the targeted tissue, preserving cell-cell communication and deposits extracellular matrix, shown to induce cellular chondrogenesis in vitro[8,9]. Additionally, cell sheets conform to the shape of the various defects, avoiding alignment issues seen in grafts such as osteochondral allograft (OCA)[10,11]. Based on adult chondrocyte sheet characterization and efficacy in animal models, autologous chondrocyte sheet clinical safety and efficacy were demonstrated on eight severe OA patients by Japanese clinical collaborator and our team[7]. However, the two-stage surgical procedure and patient individual differences remain unavoidable issues.

Our lab, Cell Sheet Tissue Engineering Center at Utah (CSTEC UTAH) is developing new allogeneic cell sheet therapies. Juvenile cartilage-derived chondrocyte (JCC) has been identified as a quality source for neocartilage regeneration due to its immune tolerant acceptability and proliferative activity. Its availability also allows chondral defect treatment in a single-stage surgical procedure. With close collaboration with a local clinical partner, we have established juvenile cartilage-derived chondrocytes (JCCs) as a prominent cell source and confirmed the safety and efficacy of engineered JCC sheets in preclinical translational research as follows[11]. JCCs exhibited stable and high growth potential in vitro over passage 10, supporting possibilities for scale-up to mass production for commercialization. JCC sheets contained highly viable, densely packed cells, showed no anchorage-independent cell growth, expressed mesenchymal surface markers, and lack MHC II expression. In nude rat focal osteochondral defect models, stable

neocartilage formation was observed at 4 weeks by JCC sheet transplantation without abnormal tissue growth over 24 weeks, in contrast to the non-treatment group showing no spontaneous cartilage repair. Regenerated cartilage was safranin-O positive, contained type II collagen, aggrecan, and human vimentin, and lacked type I collagen, indicating that the hyaline-like neocartilage formed originates from transplanted JCC sheets rather than host-derived cells.

Our study has demonstrated the safety of JCC sheets and stable hyaline cartilage formation with engineered JCC sheets utilizing a sustainable tissue supply. Cost-benefit and scaling issues for sheet fabrication and use support the feasibility of this JCC sheet strategy in clinical cartilage repair. In this symposium, we will further discuss the urgent need for future allogeneic cell-based regenerative medicine.

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Engineered Nanosystems and Nanoconjugates with Smart Functionalities for Targeted Therapy of Intractable Diseases

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Nanotechnology-based medicine (Nanomedicine) has received progressive interest in treating intractable diseases, such as cancer, and the non-invasive diagnosis through various imaging modalities. Engineered polymeric nanosystems are crucial in nanomedicine as drug carriers, gene vectors, and imaging probes[1,2]. This presentation focuses present status and future trends of nanosystems and nanoconjugates with smart functionalities for therapy of intractable diseases. Supramolecular nanosystems with 10 to 100 nm in size can prepare by programmed self-assembly of block copolymers in the aqueous entity. The most typical example is polymeric micelle (PM) with distinctive core-shell architecture. PMs have several properties relevant for nanosystems, including controlled drug release, tissue penetrating ability, and reduced toxicity[3,4].

Furthermore, smart functionalities, such as pH- and/or redox potential responding properties, can be integrated into the PM structure[5]. These smart PMs loaded with various chemotherapy reagents were proven to have a significant utility in treating intractable and metastatic cancers, including pancreatic cancer[6], glioblastoma[7,8], and tumors harboring recalcitrant cancer stem cells (CSCs)[9]. Eventually, five different formulations of the PMs developed in our group have already been in clinical trials worldwide, including Japan, Asia, the USA, and European countries[10].

Versatility in drug incorporation is another relevant feature of supramolecular nanosystems for drug delivery. Small nucleic acid (SNA)-based medicine can be self-assembled with oppositely-charged polycationic block copolymers into nanosystems through the ionic interaction[11]. In this way, siRNA- or antisense oligo (ASO)-loaded micellar or vesicular nanosystems are produced, and their utility in molecular therapy of cancer has been revealed[12-14]. Downsizing polyion complex (PIC) assembly comparable to the antibody size allowed it to cross the physiological barrier, including a thick fibrotic stroma in pancreatic cancer and the blood-brain tumor barrier in glioblastoma, exerting significant antitumor activity[15,16]. Phase I clinical trial using this small-sized PIC nanocarrier loaded with siRNA has started in Japan to treat HER2-negative breast cancer[17,18].

Furthermore, smart-nanoconjugates of checkpoint blockade antibody (anti-PDL 1 antibody) hold promise to treat intractable glioblastoma multiforme (GBM). Recently, we developed an anti-PDL 1 antibody decorated with glucosylated PEG to cross the blood-brain tumor barrier of GBM by recognizing glucose-transporter overexpressing on GBM capillaries[19]. By sensing the reductive microenvironment of GBM, PEG palisades are removed from the antibody to recover its checkpoint inhibiting ability, thereby exerting effective immune checkpoint blockade (ICB) therapy to achieve the complete remission of GBM. Notably, this ICB therapy by smart nanoconjugate successfully inducts memory effect to prevent recurrence of GBM.

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Oncolytic Adenovirus: New opportunity for targeted cancer treatment

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Oncolytic Ad, which selectively replicate in cancer, is emerging as a promising new modality for the treatment of cancer and it has several advantageous attributes over non-replicating Ads. Oncolytic Ad possesses an inherent ability to multiply, lyse infected cancer cells, and spread to surrounding cells. The potency of oncolytic Ad is drastically improved by arming it with anticancer transgenes. These transgenes can inhibit oncogenic signaling pathways as well as normalizing the hostile and dysregulated microenvironmental factors of tumor like extracellular matrix, immune surveillance, and vascularization. Most prominent aspect of oncolytic Ad-mediated therapeutic gene expression is that the gene expression is greatly amplified through viral replication and occurs in cancer-specific manner, thus leading to high level of therapeutic gene expression in tumor tissues while being absent in normal tissues.

Still, successful eradication of advanced and metastatic cancer requires systemically administrable viral vector system to efficiently target both primary tumors and metastases. As native tropism of Ad prevents successful systemic delivery of viral particles to tumor regions, we have investigated several nanomaterials for complexation with Ad to enhance the target-specific delivery of oncolytic Ad. These nanomaterials possess unique physicochemical properties that allow integration of multiple functionalities in a single design, which can be subsequently transferred to nanomaterial-complexed Ad hybrid vector system. We have demonstrated that multifunctional nanomaterial-coated Ad can be used to enhance intratumoral accumulation of Ad and restrict accumulation in nontarget tissues. Further, nanomaterial-coated oncolytic Ads is protected from hostile host environment, such as immune system and degradative enzymes, which leads to prolonged blood circulation and higher bioavailability of Ad in tumor tissues. Our findings demonstrate that efficient tumor-targeting by hybrid vector system can attenuate systemic toxicity of Ad by attenuating the hepatotoxicity, ectopic transgene expression at non-target tissues, and induction of immune response against Ad.

Akihiko Kikuchi is the professor at the Department of Materials Science and Technology, Tokyo University of Science, Japan. He received Ph.D. degree in 1992 under supervision of the late Prof. Teiji Tsuruta and Prof. Kazunori Kataoka. He was the post-doc fellow at the U. of Utah 1992-1994 in the Prof. S. W. Kim's lab. and worked on the preparation of novel polymers with sulfonylurea analogs. He then joined Tokyo Women's Medical University and worked with Prof. T. Okano until 2006, where he worked on the preparation of thermoresponsive interfaces for tissue engineering and separation of biomolecules. He then moved to the Tokyo University of Science in 2006. Since then, he is working on the preparation and characterization of various biomaterials. He was the associate editor for the Journal of Controlled Release (2009-2020) and is the Fellow, Biomaterials Science and Engineering since 2016.

Stimuli-responsive hydrogels degradable under cancer environment

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Hydrogels contain relatively large amount of water within physically or chemically cross-linked three-dimensional networks. Such character would be useful for the carriers of biopharmaceuticals, minimizing the potential risks of denaturation or loss of their biological functions. Cancer microenvironments are slightly acidic with a relatively high glutathione (GSH) concentration of approximately 10 mmol/L, compared with that in normal tissues (~3 mmol/L). Thus, hydrogels having responsive properties to local pH and reduction conditions may have potential applications as carriers for drug delivery to the cancer environment [1-4].

We have previously prepared redox-responsive trisoligo(ethylene glycol) (trisOEG) hydrogels connected with disulfide bonds [5]. When disulfide bonds are disrupted in the presence of dithiothreitol, proteins loaded in the hydrogels are rapidly released. To introduce other functions rather than redox-responsive property, we aimed to prepare multi-stimuli-responsive hydrogels in one pot through the epoxy ring opening reaction between poly(ethylene glycol) diglycidyl ether (PEGDE) and cystamine (CA) [6].

By changing the mixing condition of PEGDE and CA and PEGDE concentrations, we successfully prepared three-dimensionally cross-linked hydrogels with PEGDE:CA= 1.5:1.0 to 2.0:1.0 (molar ratio) and PEGDE of more than 20 wt.%. Thus, we used the hydrogels prepared with PEGDE:CA of 2.0:1.0 with PEGDE concentration of 30 wt.%. As hydrogels contained either secondary or tertiary amines arising from CA units, pH-responsive swelling change was observed; below pH 6.5 abrupt swelling was indicated at 37°C. This suggested the pH responsive property of the hydrogels under cancer acidic environment. Furthermore, thermoresponsive swelling change was observed for the prepared hydrogels. Degradation behavior of the hydrogels was then investigated in the presence of 3mmol/L dithiothreitol (DTT) as an accelerated test. The hydrogels showed gradual size change at 40 min., followed by complete degradation and dissolution after 200 min. incubation in DTT containing phosphate buffer saline solution through the disruption of disulfide bonds in CA units. Changes in weight remaining in the presence of 3 mmol/L DTT indicated that linear decrease in gel weight for 200 min. incubation. Swelling ratio of the hydrogels, however, decreased initially and during 40 min. to 80 min., remained constant value, whereas the remaining weight changed from 80% to 60% at the same time intervals. The result suggested that the hydrogels degraded starting from near the hydrogel surface at 37°C where hydrogels existed dehydrated state. After 80 min, the swelling ratio increased with time due to the reduced cross-linking points of the hydrogel network through degradation of disulfide bonds. After complete degradation, sample solution was examined by gel permeation chromatography, and results indicated that remaining materials have molecular weight below 1,000, which corresponds the PEGDE with CA segments at both ends.

The protein loaded hydrogels were then prepared by incubation of hydrogels in protein solution at 5°C and then increased to 37°C. Loading amount was dependent on the molecular weight of the proteins. At pH 5.4, the hydrogels existed slightly swollen condition, only a small amount of proteins released from the hydrogels. This may be due to the electrostatic interaction between proteins and hydrogels. However, in the presence of reducing agent, proteins completely released

within 1 h. Thus the protein drugs can be released only under reduction condition. We recently elucidated that the hydrogels degraded in the presence of HeLa cell cultures, suggesting that the increased reduction condition (due to the production of GSH by the HeLa cells) surrounding the cancer cells leads the hydrogel degradation. These results suggested that the prepared hydrogels may be used for treatment of cancer that remained after surgery.

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BIORESPONSIVE POLYMERSOMES FOR CANCER THERAPY AND IMMUNOTHERAPY

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Polymersomes with good stability and great versatility are an interesting alternative to liposomes for controlled drug delivery. In the past years, we have developed disulfide-crosslinked biodegradable polymersomes and chimaeric polymersomes based on proprietary dithiolane trimethylene carbonate (DTC) technology, which mediate efficient loading and targeted intracellular delivery of different bioactives including chemotherapeutics [1], molecular targeted drugs, peptides and proteins [2], nucleic acids [3] and immunoadjuvants [4]. DTC-based polymersomal nanoformations are easy to make and robust while will quickly release payloads after transporting into cytosols of target cells as a result of de-crosslinking of polymersomal membrane. Notably, our recent studies show that chimaeric polymersomes quantitatively co-encapsulating lysates of tumor cells and adjuvants can home to lymph nodes and trigger strong and durable immune responses, leading to a high complete remission (CR) rate in different murine tumor models. CpG-loaded apolipoprotein E peptide-functionalized chimaeric polymersomes mediate strong immunotherapy of orthotopic murine glioma in mice by systemic administration. The disulfide-crosslinked biodegradable polymersomes and chimaeric polymersomes are unique and have a high potential for cancer therapy and immunotherapy.

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Activatable Imaging Probe for Receptor-Ligand Binding

Ick Chan Kwon

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For decades, molecular imaging, capable of monitoring intercellular/intracellular molecular processes in organisms, has provided valuable information for a variety of research fields. Biomarkers such as enzymes, receptors, and proteins can provide information for early diagnosis of diseases and monitoring of therapeutic effects, and thus can be used as targets for molecular imaging. Among them, molecular imaging technology based on receptor-ligand interaction is emerging as a promising strategy for monitoring intractable diseases such as cancer. However, the basic requirement for this kind of imaging probe is to provide disease-specific information along with high imaging sensitivity. Here, we developed a self-quenching imaging probe capable of emitting fluorescence (activation) via a de-quenching reaction after internalization via receptor-ligand binding. Demonstrations of EGFR or CD47 target specific fluorescence signals will be presented in this presentation.

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Degradation-regulated Architecture of Injectable Smart Hydrogels Enhances Humoral Immune Response and Potentiates Antitumor Activity in Human Lung Carcinoma

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Cancer vaccines that elicit a robust and durable antitumor response show great promise in cancer immunotherapy. Nevertheless, low immunogenicity and weak immune response limit the application of cancer vaccines. To experience next generation cancer vaccines that elicit robust, durable, and anti-tumor T cell response, herein we design injectable smart hydrogels (ISHs) that self-assemble into a cellular microenvironment like microporous network using a simple hypodermic needle injection, to localize the immune cells and program host cells. ISHs, composed of levodopa- and poly(ε-caprolactone-co-lactide) ester-functionalized hyaluronic acid (HA-PCLA), are loaded with immunomodulatory factor (OVA expressing plasmid, pOVA)-bearing nano-sized polyplexes and granulocyte-macrophage colony-stimulating factor (GM-CSF) as dendritic cell (DC) enhancement factor. Subcutaneous administration of ISHs effectively localized immune cells, and controlled the delivery of immunomodulatory factors to recruit immune cells. The microporous network allowed the recruitment of a substantial number of DCs, which was 6-fold higher than conventional PCLA counterpart. The locally released nano-sized polyplexes effectively internalized to DCs, resulting in the presentation of tumor-specific OVA epitope, and subsequent activation of CD4+ T cells and generation of OVA-specific serum antibody. By the controlled release of nano-sized polyplexes and GM-CSF through a single subcutaneous injection, the ISHs effectively eliminated B16/OVA melanoma tumors in mice. These ISHs can be administered using a minimal invasive technique that could bypass the need for extracorporeal training of cells ex vivo, and provide sustained release of cancer vaccines for immunomodulation. These important findings suggest that ISHs can serve as powerful biomaterials for cancer immunotherapy.

Dr. Christine Allen is the Associate Vice-President and Vice-Provost, Strategic Initiatives at the University of Toronto and a full professor in the Leslie Dan Faculty of Pharmacy. She is the CEO and Scientific Director of the Nanomedicines Innovation Network, a National Centre of Excellence funded by the Government of Canada. Her research focuses on the design of innovative materials and drug delivery technologies with over 150 publications in this area. She is the co-founder and served as President of Nanovista Inc., a company focused on high-precision, image-guided cancer therapy. Professor Allen is a fellow of the American Institute for Medical and Biological Engineering, the Controlled Release Society (CRS) and the Canadian Academy of Health Sciences. She is the Editor-in-Chief of the Journal of Controlled Release, the President-Elect of the CRS and an appointed member of the Governing Council of the Natural Sciences and Engineering Research Council of Canada.

Harnessing Automation and Machine Learning for Sustainable Drug Formulation Development

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The ongoing COVID19 pandemic has highlighted the importance of drug formulation in the healthcare sector. The use of lipid-based nanotechnology has made RNA-based vaccines clinically viable by conferring protection and targeted delivery to mRNA cargo. There is no question that nanomedicines have incredible potential to unlock new therapies for many other chronic and infectious diseases. Despite the great potential of nanomedicines, their design and development are far from trivial. It has been noted that many drug delivery systems reported as of late are overly complicated, in the sense that novelty and the desire to be published can trump translational feasibility and commercial viability. As a result, there is a prominent gap between the volume of formulations reported in the literature and candidate formulations that truly hold clinical promise. While this is a very exciting time to be a researcher developing nanomedicines, we are also very privileged to be living in countries with free access to medical advances (such as mRNA vaccines), and to have access to the necessary funding to conduct this research. We have a duty to ensure that we are conducting our research in a responsible and sustainable way.

The integration of technological advances such as artificial intelligence and experimental automation can improve the sustainability of formulation design and development. The Allen Lab has recently begun to apply these technologies with the goal of informing decision making early in the formulation development process. Not only does the combination of automation and artificial intelligence tackle issues of data reproducibility, but it can accelerate the development of new advanced drug delivery systems, therefore improving access to effective medicines and increasing patients' quality of life.

Alexander Kabanov, a Distinguished Professor at the Eshelman School of Pharmacy, UNC-Chapel Hill, director of UNC Center for Nanotechnology in Drug Delivery, Carolina Institute for Nanomedicine, and NCI's T32 training program in Cancer Nanotechnology. Professor of Chemistry at Moscow State University. Graduated from the Moscow State University in 1984, where also received Ph.D. in 1987 and D.Sc. in 1990. Made broad impact to nanomedicine by introducing polymeric micelles, polyelectrolyte complexes, nanogels and exosomes for the therapeutic delivery of small drugs, nucleic acids, and proteins. His work led to first polymeric micelle drug to enter clinical trials. Highly Cited Researcher in Pharmacology and Toxicology. Published >350 scientific papers (>43,500 citations, Google h-index 106), holds 37 US patents and co-founded several companies. Trained >70 graduate students and postdocs half of whom are women and underrepresented minorities. Founded Nanomedicine and Drug Delivery symposium series (www.nanodds.org), chaired Gordon Research Conferences. Recipient of Russian Megagrant in 2010 and Member of Russian Grants Council. Honors include Lenin Komsomol Prize, NSF Career award, and George Gamow award. Elected to AAAS, NAI, Academia Europaea, Russian Academy of Sciences, AIMBE and CRS Colleges of Fellows. Past President of Russian American Science Association and director-at-large, CRS.

Morphology, Partitioning and Pharmacological Performance in Block Copolymer Systems

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Poly(2-oxazoline) (POx) based polymeric micelles (PM) display unprecedented high loading with respect to water-insoluble drugs. Such PMs greatly enhance the solubility and stability of drugs improve their efficacy and safety in a transformative way. We will discuss the effect of the micelle morphology on drug pharmacological performance in the spherical and worm-like particles. PMs can elongate over time from spherical to worm-like structure, depending on the amount and type of drug. Small spherical micelles rapidly accumulate in tumors while carrying more drug than worm-like micelles that accumulate slower and release drug in the blood where it gets cleared. As a result, greater anti-tumor effects are seen with spherical micelles. The dynamic character of drug–micelle species and control of micelle morphology play critical role in the drug delivery in tumors Reference: https://doi.org/10.1101/2021.06.10.447962. Funding: NIH CA198999, CA264488. Conflict of interest: DelAQUA Pharmaceuticals (co-founder, shareholder, officer).

Glen Kwon is the Jens T. Carstensen Distinguished Professor in Pharmaceutical Sciences in the School of Pharmacy at University of Wisconsin. His research in pharmaceutics focuses on chemotherapy and peptide biologics, interested in drug targeting and long-acting injectables for cancer and fibrosis. Dr. Kwon received a Ph.D. in Pharmaceutics from Department of Pharmaceutics in College of Pharmacy, University of Utah. Professor Sung Wan Kim was Chair of the Supervisory Committee. Dr. Kwon was a Japan Society for Promotion of Science Post-Doctoral Fellow at International Center for Biomaterials Science, Institute of Biomedical Engineering at Tokyo Women's Medical College and Assistant Professor at the Faculty of Pharmacy and Pharmaceutical Scientists at the University of Alberta, Canada. He received Jorge Heller Journal of Controlled Release/Controlled Release Society (CRS) Outstanding Paper Award for his post-doctoral research that evidenced the EPR effect for a polymeric drug-micelle. He served on the Scientific Advisory Board of Nanocarrier Inc., Tokyo, Japan. He is a Fellow of American Association of Pharmaceutical Scientists (AAPS). He is a member of the editorial board of Journal of Controlled Release; UW Carbone Cancer Center Experimental Therapeutics Program and Wisconsin Center for NanoBioSystems. Dr. Kwon is co-founder of Co-D Therapeutics Inc., Madison, WI.

PEGylated Functional Upstream Domain Peptide from *S. Pyogenes* Disrupts Fibronectin Fibrillogenesis and Reduces Bleomycin-Induced Pulmonary Fibrosis

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Fibrous is a disease of aberrant wound healing and tissue repair, characterized by excessive deposition of extracellular matrix (ECM), including collagen. Fibrosis is an outcome of chronic inflammatory diseases and leads to organ failure and *ca*. 45% of deaths in the developed world. Nintedanib and pirfenidone, two approved antifibrotic drugs, do not halt progression of fibrosis. Disruption of fibronectin (FN) fibrillogenesis by FN genetic ablation reduces fibrosis *in vivo*. Alternatively, a Functional Upstream Domain (FUD) peptide, derived from F1 adhesion protein of *S. pyogenes*, tightly binds *N*-terminal 70 kD domain of FN1, interrupts FN fibrillogenesis and reduces fibrosis *in vivo*. However, like most peptides, FUD absorbs rapidly after subcutaneous (SC) injection ($t_{1/2}$=1 hr) and is rapidly cleared from plasma. PEG has been conjugated on the *N*-terminus of recombinant FUD peptide, and its SC absorption is slower ($t_{1/2}$= 6.8 hr). $PEG_{20,000}$-FUD peptide retains high affinity for FN (K_d=10 nM) and preferentially localizes in injured lungs of bleomycin-treated mice, confirming FN target expression and engagement. $PEG_{20,000}$-FUD peptide (12.5 mg/kg/day; QDx10) reduces hydroxyproline in lungs of bleomycin-treated mice compared to $PEG_{20,000}$-*m*FUD peptide. No adverse reactions were noted. In summary, *in vitro* and *in vivo* results show that $PEG_{20,000}$-FUD peptide disrupts FN fibrillogenesis and collagen deposition, suggesting a safe and effective treatment for fibrotic disease.

Twan Lammers obtained a D.Sc. in Radiation Oncology from Heidelberg University in 2008 and a Ph.D. in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at RWTH Aachen University. In 2014, he was promoted to full professor of medicine at RWTH Aachen University Clinic. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided (theranostic) drug delivery systems are being developed, as well as materials and methods to monitor tumor growth, angiogenesis, inflammation, fibrosis and metastasis. He has published over 250 papers (17000 citations, h-index 75), and received multiple scholarships and awards, including a starting and consolidator grant from the European Research Council, the Young Investigator Award of the Controlled Release Society, the Adritelf International Award, and the Belgian Society for Pharmaceutical Science International Award. He is on the editorial board of several different journals, and serves as a handling editor for the Journal of Controlled Release, Drug Delivery and Translational Research, and Molecular Imaging and Biology. Since 2019, he is included in the Clarivate Analytics list of Highly Cited Researchers.

Theranostic Strategies to Promote Polymeric Nanomedicine Clinical Translation

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Polymeric nanomedicines are extensively used to improve the efficacy and reduce the toxicity of chemotherapeutic drugs. Nanomedicine formulations traditionally rely on the "EPR" effect for target site accumulation, which is highly variable, both in animal models and in patients[1]. To tackle heterogeneity in target site accumulation, and to improve the performance and translation of polymeric nanomedicines, we are working on materials and methods to monitor and modulate tumor-targeted drug delivery[2]. In the present lecture, recent progress on several of these efforts will be summarized, including (1) imaging-based analysis of EPR effect dynamics during nano-taxane treatment with theranostic polymeric micelles[3]; (2) correlation analysis of polymeric micelle tumor targeting with therapeutic outcome (Fig. 1A-C)[3]; (3) imaging-based analysis of polymeric micelle targeting to metastases in mouse models and cancer patients; and (4) recent evidence that histopathological assessment of tumor tissue biopsy biomarkers can be used for cancer nanomedicine patient stratification. The insights obtained provide a rational basis for promoting polymeric nanomedicine clinical translation.

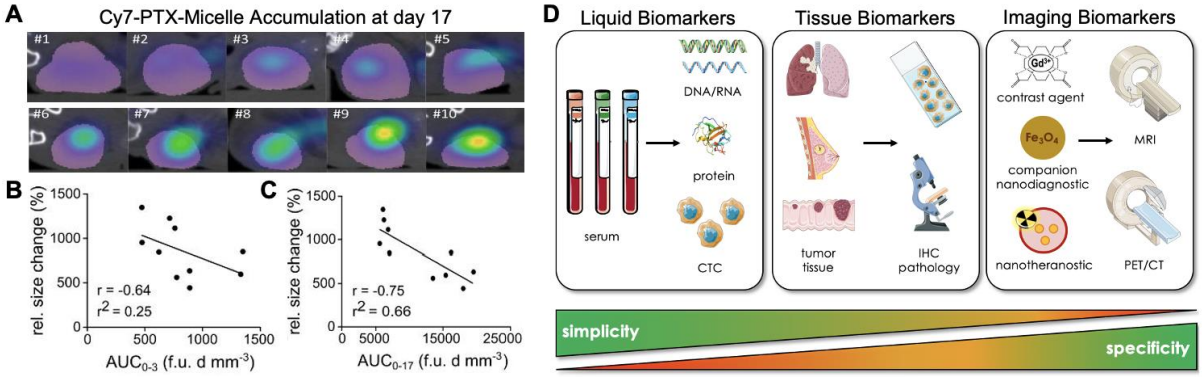

Figure 1: Imaging and biopsy biomarkers for cancer nanomedicine patient stratification. A: The tumor accumulation of polymeric micelles loaded with paclitaxel and labeled with Cy7 was monitored multiple times during 3 weeks of treatment, showing that higher levels of micelle accumulation correlate with better treatment outcome[3]. B-C: Monitoring micelle tumor accumulation once (B: on day 3, AUC_{0-3}, in fluorescence units per day per cubic millimeter) less accurately predicts tumor response than monitoring micelle accumulation multiple times during 17 days (C: AUC_{0-17})[3]. D: Schematic depiction of the specificity vs. simplicity of biomarkers that can be used for cancer nanomedicine patient stratification[2].

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Dr. You Han Bae is Distinguished Professor in Pharmaceutics and Pharmaceutical Chemistry at the University of Utah and the founder of Ileo Science, Inc. His expertise includes responsive polymers and their biomedical applications and novel delivery systems for proteins, genes, and small molecules. In these fields, he has published more than 300 research articles/book chapters/patents (Google Scholar: citations ~ 40,000 with a h-index of 96) and been recognized as one of Highly Cited Researchers each year for the period of 2014-2018 (by Thomson Reuters & Clarivate Analyatics) in the field of pharmacology. He is an elected fellow of AIBME, AAPS, and CRS. He is currently interested in oral nanoparticle absorption and drug delivery.

Mechanistic pathway of nanoparticle ileal absorption

Feiyang Deng, Kyoung Sub Kim, Hana Cho, Ji Young Moon, and You Han Bae

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Solid nanoparticles (GCPN) from carboxylated polystyrene (CPN, ~100 nm) of which surface was conjugated with a select bile acid, glycocholic acid (GCA) having a lowest log P among bile acids, interacted with cells expressing apical sodium dependent bile acid transporter (ASBT) [1]. GCPN was internalized into and expelled from the cells of SK-BR-3 (a human breast cancer cell line) and Caco-2 (a human colon cancer cell line). The interactions supported oral bioavailability as high as ~47% of GCPN in mice and rats [1] and significantly improved oral bioavailability of self-assembled nanoparticles which carry active pharmaceutical ingredients (API) [2-5]. The PK profiles were flatter than those from other administration routes and GCPN was absorbed to the intestinal lymphatic system bypassing the hepatic first-pass. Fasting animals before 4 hours and after one hour oral administration of GCPN allowed the highest oral bioavailability [6]. This strongly suggests the feasibility of a new platform for oral delivery of various classes of API, including biologics [7].

This presentation summarizes experimental results of mechanistic investigation for the steps of GCPN transportation in Caco-2 cells. For a close look at the endocytosis process, confocal imaging, pharmacological inhibitors, silencing characteristic proteins in specific endocytoses, and tracing gene expression levels before and after GCPN treatment were employed. Inhibitions of membrane mobility, dynamin, caveolae pinching, and actin polymerization negatively impacted GCPN uptake by the cells; inhibitions of clathrin-mediated endocytosis and cholesterol activity did not change the uptake rate. The transcytosis of GCPN may occur via endoplasmic reticulum (ER)-Golgi pathway, not through the endosome/lysosome route. CPN passes through the ER-Golgi Complex-lysosome. Interestingly GCPN was slower in exocytosis than CPN inside cells by downregulating various genes associated with transcytosis. IBABP (ileal bile acid binding protein), that binds bile acids and cross the cell cytoplasm to OSPa/b on the basolateral membrane for pumping out bile acids, seemed colocalize with GCPN. It is not clear IBABP plays any role for GCPN to avoid the endosome/lysosome pathway. The transcytosis and exocytosis of GCPN resemble those for chyromicrons (CM) and GCPN promotes lipid uptake and transport as GCA did, but are not linked to OSPa/b. The mechanistic pathways need further investigation and the knowledge may help the design of oral NP carriers, especially for nucleotide delivery.

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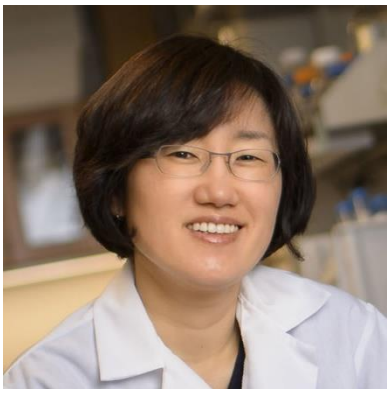

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Local delivery of paclitaxel and nucleic acids via an immunoactive polymer for systemic therapy of solid tumors

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Since the success of immune checkpoint blockade and chimeric antigen receptor T cell therapy, over a dozen immunotherapies have been approved in the past few years, and thousands of immunotherapeutics are currently in the development pipeline. However, immunotherapy benefits only a small fraction of cancer patients with identified tumor antigens and/or well-accessible tumors. New therapeutic strategies are needed to improve the efficacy of immunotherapeutics in broader patient populations with hard-to-reach, unidentified tumors. An approach gaining interest in the immuno-oncology community is to treat locatable and accessible tumors locally and stimulate antitumor immunity *in situ* to exert systemic effects against distant tumors. The rationale of local immunotherapy is that properly treated tumor cells serve as a depot of tumor antigens and induce systemic immune response against tumor via circulating immune cells. By confining therapeutics in tumors, local immunotherapy can avoid systemic side effects, such as immune-related adverse events, which have limited the utility of traditional approaches.

For effective local immunotherapy, several events need to be coordinated coherently, including *in-situ* generation of tumor-associated antigens (TAAs), activation of antigen-presenting cells (APCs), infiltration of immune cells to the tumor microenvironment (TME), and the maintenance of immunoactive TME. The complexity of antitumor immune responses requires combinations of agents with distinct mechanisms of action. For example, chemotherapeutic drugs are used to induce immunogenic cell death to generate TAAs and release damage-associated molecular patterns, which make the dying cells vulnerable to APC uptake. Nucleic acids and nucleotides are frequently employed due to their diverse functions: small nucleotides can serve as potent immunoadjuvants, and siRNA can be used to block immune checkpoints.

For local delivery of multiple immunotherapeutics in cancer therapy, carrier selection is important in at least three aspects. First, a carrier can help retain immunotherapy locally to maximize pharmacological effects of therapeutic agents in tumors and prevent their systemic side effects. Second, a carrier can ensure the colocalization of multiple agents, which share little physicochemical features and would otherwise be difficult to co-deliver. Third, a carrier engineered with an immunoadjuvant function can play an active role in triggering antitumor immunity, synergizing with immunostimulatory effects of therapeutic drugs.

In our recent study, we have developed a new carrier of immunotherapeutics for local application, based on an amphiphilic modification of polyethyleneimine (PEI), a polymeric gene carrier and a toll-like receptor-5 agonist. We conjugated lithocholic acid (LCA), a hydrophobic bile acid with an immunostimulatory effect on APCs, to the flank of PEI to accommodate hydrophobic drugs and enhance the immunoadjuvant function. We demonstrate that the PEI-LCA conjugate (2E') (**Fig. 1a**) forms supramolecular assemblies in water, which load hydrophobic drugs and nucleic acids by simple mixing (**Fig. 1b**), and stimulates APCs to complement the activities of the active ingredients (**Fig. 1c**). A single intratumoral administration of 2E', along with paclitaxel (PTX), induces immediate regression of tumors and generates systemic immunity in the CT26 tumor model. Additional incorporation of siRNA targeting PD-L1 (siPD-L1) or cyclic

dinucleotide (CDN) further enhances the immunostimulatory effects, leading to the regression of large established tumors and tumor-free survival in CT26, B16F10, and 4T1 tumor models after a single administration (**Fig. 2**). The local induction of antitumor immunity (**Fig. 3**) activates systemic antitumor immunity (**Fig. 4**) and immune memory to protect surviving animals from tumor rechallenge and metastasis. The potent antitumor activity of this immunoactive complex demonstrates the importance of a rationally-designed drug carrier and supports the feasibility of treating tumors systemically by local immunotherapy.

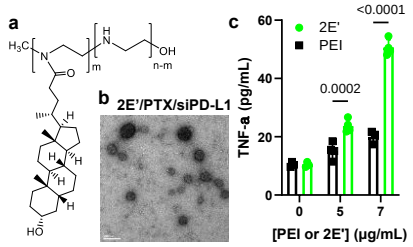


Fig. 1. (a) Structure of PEI-LCA conjugate (2E'); (b) Transmission electron microscopic image of 2E'/PTX/siPD-L1 (1:0.2:0.67, w/w/w); (c) TNF-α secretion by bone-marrow derived dendritic cells (BMDC) after incubation with 2E'

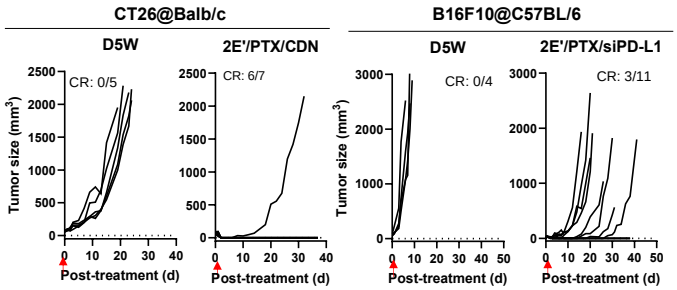


Fig. 2. Individual growth of tumors treated with D5W (5% dextrose,

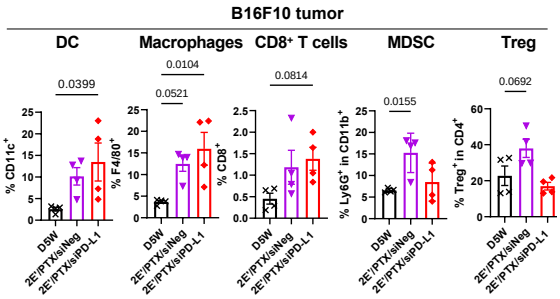


Fig. 3. Effects of a single intratumoral injection of 2E'/PTX/siPD-L1 on immunophenotype of B16F10 tumors. DC: dendritic cells; MDSC: myeloid-derived suppressor cells; Treg: regulatory T cells; siNeg: negative control

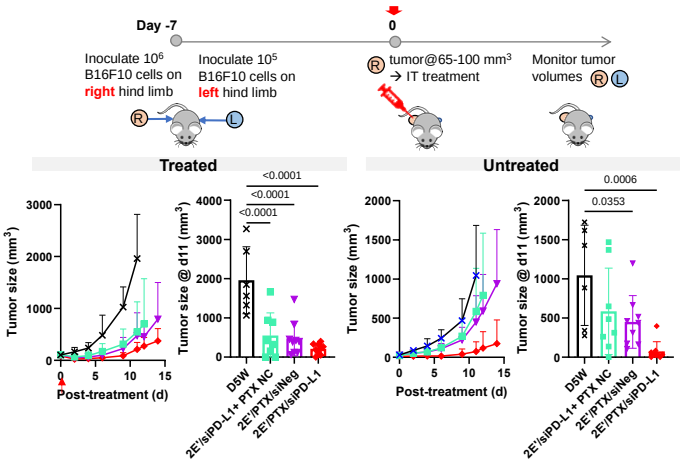
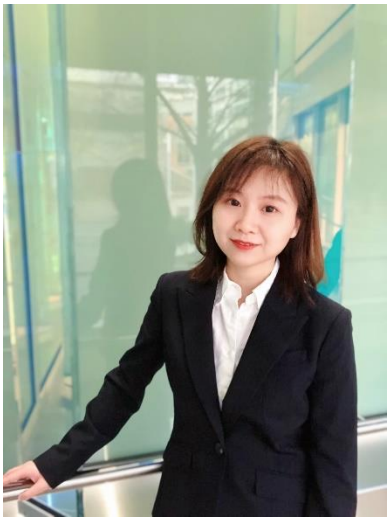


Fig. 4. Effects of a single intratumoral injection of 2E'/PTX/siPD-L1 on systemic anti-tumor effect: (Top) Schedule of bilateral B16F10 tumor inoculation in C57BL/6 mice and treatment injection; (bottom) tumor growth curves and tumor sizes on day 11 post-treatment of treated and untreated tumors after a single treatment of D5W (n=6), 2E'/siPD-L1 + PTX NC (n=8), 2E'/PTX/siNeg (n=9) or 2E'/PTX/siPD-L1 (n=9); mean±SD. PTX NC: PTX nanocrystals with activity equivalent to Abraxane. p-values were calculated by Dunnett's multiple comparisons test following one-way ANOVA. Note that some of the 2E'/PTX/siPD-L1-treated mice were euthanized due to tumor ulceration despite the small



Dr. Yue Lu is an Assistant Professor in the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. She received her B.S. degree in Chemistry from Nanjing University, and Ph.D. at the University of North Carolina at Chapel Hill and North Carolina State University, under the guidance of Dr. Zhen Gu in the Joint Department of Biomedical Engineering. She completed her postdoctoral fellowship with Dr. James R. Heath at the Institute for Systems Biology and Caltech. The Lu Digital Pharmaceutics (DigiPharma) Laboratory aims to develop and apply state-of-the-art technologies to understand disease pathologies, and develop treatment strategies for cancer, infectious diseases, and autoimmune diseases. Dr. Lu has authored 28 journal articles, including first-author publications in Nature Reviews Materials, Nature Communications, PNAS, Nano Letters, Journal of Controlled Release and Chemical Communications. She is the recipient of the Graduate Research Advances in Delivery Science Award from the Controlled Release Society, the Chinese Government Award for Outstanding Self-financed Students Abroad, and the Distinguished Graduate Dissertation Award from NC State College of Engineering.

Digital Pharmaceutics: A data-driven approach for precision medicine

Yue Lu

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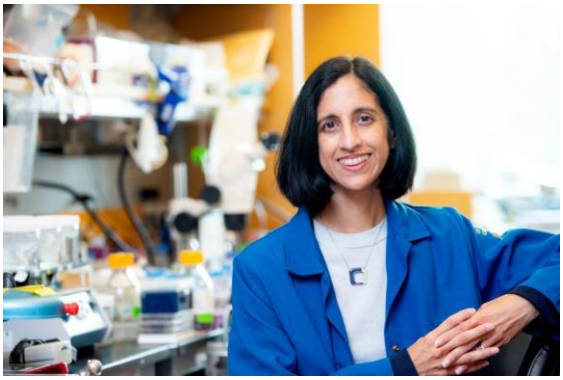
The Lu Digital Pharmaceutics Laboratory at the University of Utah aims to develop and apply state-of-the-art technologies to understand disease pathologies, and develop treatment strategies for cancer, infectious diseases, and autoimmune diseases. In this talk, I will first provide an overview of our research capabilities and recent efforts in precision medicine. I will then focus on an analysis framework designed to uncover integrated pathophysiological and molecular features of the tumor microenvironment. This framework uses spatially resolved proteomics, fluorescent microscopy image analysis, and data-driven computational models to determine the correlates of tumor proliferation, CD8 infiltration, and treatment response. We applied this approach to study patients with either recurrent glioblastoma multiforme or high-risk melanoma, who were treated with neoadjuvant immune checkpoint blockade



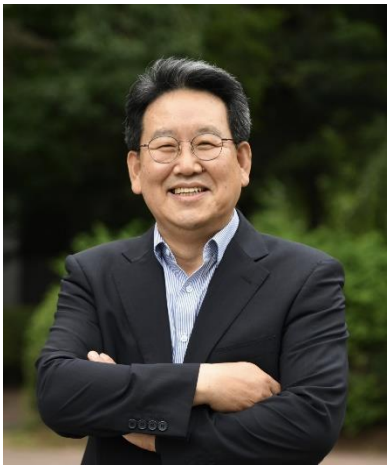
Tejal Desai is the Deborah Cowan Endowed Professor of the Department of Bioengineering & Therapeutic Sciences, Schools of Pharmacy and Medicine at University of California, San Francisco (UCSF); and Professor in Residence, Department of Bioengineering, UC Berkeley (UCB). She serves as director of the NIH training grant for the Joint UCSF/UCB Graduate Program in Bioengineering, and founding director of the UCSF/UCB Masters Program in Translational Medicine. She is also the Inaugural Director of the UCSF Engineering and Applied Sciences Initiative known as HIVE (Health Innovation Via Engineering). Starting in September 2022, she will be starting at Dean of Engineering at Brown University. Desai's research spans multiple disciplines including materials engineering, cell biology, tissue engineering, and pharmacological delivery systems to develop new therapeutic interventions for disease. She seeks to design new platforms, enabled by a advancements in micro and nanotechnology, to overcome existing challenges in therapeutic delivery. She is President of the American Institute for Medical and Biological Engineering and is a fellow of AIMBE, IAMBE, CRS, and BMES. In 2015, she was elected to the National Academy of Medicine and in 2019 to the National Academy of Inventors. Desai is a vocal advocate for equity and inclusion and her work to break down institutional barriers to equity and cultivate a climate of inclusion was recognized by the AWIS Judith Poole Award and the UCSF Chancellors Award for the Advancement of Women. As president of AIMBE, she has led advocacy efforts for increased scientific funding and addressing workforce disparities in science/engineering. She received her B.S. from Brown University in biomedical engineering in 1994 and was awarded a Ph.D. in bioengineering jointly from UCSF and UC Berkeley in 1998.

Nanostructured Interfaces to Modulate Epithelial Transport

Tejal Desai

University of California, San Francisco

Therapeutic peptides, proteins, and macromolecular drugs represent highly efficacious approaches for the pharmacologic treatment of human diseases. However, current administration of such drugs continues to be bolus injection which is inconvenient, painful and can lead to variable dosing. Over the years, we have identified classes of nanostructured surfaces that enhance the permeability of macromolecules across epithelial cells in response to direct contact. In this talk, I will discuss the molecular mechanisms through which epithelial permeability is modulated including alterations in tight junction remodeling, cell polarity, protein translocation, and transcytosis. Roles for integrin engagement induced by nanotopography that promote epithelial permeability will also be discussed. The understanding on nanostructure-induced changes in epithelial permeability will enhance our understanding of cell-material interactions at the nanoscale and inform the development of new drug delivery systems.



Ki Dong Park, Ph.D, FBSE Ki Dong Park received his B.S. and M.S. at Hanyang University, Korea, and Ph.D. in Pharmaceutics and Pharmaceutical Chemistry at the University of Utah (under Professor Sung Wan Kim), USA in 1990. After a postdoctoral training at CCCD in Utah, he worked as a principal research scientist at Korea Institute of Science and Technology from 1991 to 2000. He joined the faculty in the Department of Molecular Science and Technology at Ajou University in 2000. He served as President of the Korean Biomaterials Society in 2013. He is a Fellow of Biomaterials Science and Engineering of the International Union of Societies of Biomaterials Science and Engineering. He is working as conference chair of World Biomaterials Congress (WBC2024) to be held in Korea, 2024. He is a Fellow of Korean Academy of Science and Technology. He also received many awards including National WOONGBI Medal for his contributions to scientific achievements. He has published over 310 scientific articles, many book chapters and possessed 60 patents. His research interests are wide-ranging from implants, controlled drug delivery, regenerative medicine, and biomimetic surface modification.

Injectable Hydrogels Releasing Reactive Oxygen/Nitrogen Species for Tissue Regeneration

Ki Dong Park

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Reactive oxygen and nitrogen species (RONS) are generated in cellular metabolism and have been indicated as critical modulators for various therapeutic applications, such as treatment of vascular disorders, wound healing, and cancer treatment. However, the short half-life and "double-edged sword" functions of RONS in living systems are great challenges to its clinical applications. Therefore, the development of efficient carriers to deliver RONS at physiological conditions to the targeted sites is highly desirable. Recently, injectable hydrogels have been widely used as bioactive materials for the controllable and local delivery of therapeutic agents for tissue regeneration, owing to their extracellular matrix mimicking properties, tuneable mechanical properties and minimally invasive surgical procedure. In our lab, we developed injectable gelatine hydrogels that can control RONS (H_2O_2, NO) release for a wide range of possible applications, including wound healing, vascular disorders, anti-infection, and anti-senescence. In our approach, phenol moieties were conjugated onto the gelatine backbone to enable crosslinking of them for hydrogel formation via enzymatic crosslinking reaction of horseradish peroxidase (HRP). The physico-chemical properties of hydrogels, including gelation time, mechanical strength, and degradation rate are easily controlled by varying concentrations of HRP and H_2O_2. Glucose oxidase (GOx) or copper nanoparticles (Cu NPs) was incorporated into hydrogels matrices to produce H_2O_2 and NO, respectively. The *in vitro* release studies demonstrated that the release behaviour of H_2O_2 and NO from the hydrogel matrices can be precisely controlled in a wide range of concentrations (from nano- to micromol). The hydrogels with optimal conditions enhanced the cellular activities of endothelial cells (proliferation, migration, and tube formation), facilitated neovascularization, improved the regenerative capacity of stem cells, or inhibited the bacterial growth. Our results suggest that RONS-releasing gelatine hydrogels can be utilized as advanced materials for tissue regenerative medicine and 3D bioprinting applications.

Patrick S. Stayton Distinguished Career Professor Department of Bioengineering, University of Washington Patrick Stayton serves as the Distinguished Career Professor in the Department of Bioengineering at the University of Washington. Dr. Stayton is the founding Director of the Institute for Molecular Engineering and Sciences (www.moles.washington.edu). His laboratory develops new materials for application to unmet medical needs in the therapeutics, diagnostics, and regenerative medicine fields. He has published over 300 scientific papers. Dr. Stayton has a strong interest in translating the group's research, has been awarded many patents, and is a co-founder of several startup companies, including PhaseRx Inc. that developed RNA drug delivery technologies. Dr. Stayton has been elected as a Fellow of the American Institute for Medical and Biological Engineering, and has been the recipient of the Clemson Award from the Society For Biomaterials and the CRS-Cygnus Recognition Award from the Controlled Release Society. He served as Co-Chair of the Gordon Conference on Drug Carriers in Medicine and Biology in 2010. He has also been awarded the College of Engineering's Faculty Innovator Award, and the Distinguished Teacher and Mentor Award from the Department of Bioengineering

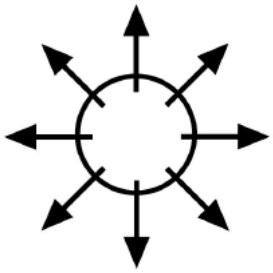
Engineering Prodrug Therapies For Infectious Disease Therapy

Patrick Stayton

Professor, Department of Bioengineering & Molecular Engineering & Sciences Institute, University of Washington, Seattle WA USA

A polymeric prodrug platform has been developed for infectious disease therapeutics. The "drugamers" can be designed to improved drug targeting and with controlled pharmaco-kinetic profiles. The polymeric prodrugs have been initially developed against pulmonary infections, where they have shown excellent activity against highly lethal bacterial disease models. The platform has been further broadened to include other pulmonary infectious disease therapy including TB and antiviral therapeutics against Covid. In addition, this platform has been extended to target liver-stage malaria therapy, using GalNAc targeting of hepatocytes to improve the therapeutic index of radical cure drugs such as tafenoquine. Finally, the polymeric prodrugs are also showing promise as a new long-acting depot product for HIV prophylaxis with anti-viral drugs. Recent advances across these applications will be covered.

POSTER ABSTRACTS



Recombinant anti-PD-1-immunotoxin-DT for autoimmune disease treatment

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Salt Lake City, Utah 84124 United States

There are more than 23.5 million Americans who are suffering from autoimmune diseases (ADs). Furthermore, no effective treatments have been found to cure ADs. One common treatment is to use immunosuppressive medicines such as corticosteroids, which can alleviate the severity of damage caused by autoimmune attack. However, severe infections or cancers can occur when using those suppressive drugs for a long term. Thus, there is an urgent need in investigating more effective therapies. Nowadays new therapies that target immune cells, such as belatacept, provides a promising way to treat autoimmune diseases. Belatacept is effective in inducing immune tolerance through CTLA-4 pathway thus represses the autoimmunity. However, considering the complexity of autoimmune diseases, it's insufficient to just research one marker protein. Previous studies have shown that Programmed Death 1 (PD-1) proteins are inducibly expressed upon activation of lymphocytes (including activated T and B cells). Hence it is possible to specifically target activated immune cells without affecting naïve immune cells. This selection reduces the possibility of occurrence of lymphopenia, which is a common side effect in the current AD treatments. The strategy mainly has two advantages, on the one hand, the immunotoxin won't severely weaken the immune system since almost all the naïve T and B cells won't be affected given that they do not express PD-1 protein, on the other hand, it can eliminate both activated T and B cells which would result in the improved efficacy of treatment.

We designed an anti-PD-1-immunotoxin-DT that specifically targets PD-1 and eliminates activated pathogenic immune cells. The design strategy is based on diphtheria toxin (DT). DT is composed of one catalytic domain and one targeting domain. By genetically changing the targeting domain into anti-PD-1 single chain variable fragments (scFvs), we can potentially take advantage of anti-PD-1-immunotoxin-DT to specifically target PD-1-positive activated lymphocytes and eliminate them by the catalytic domain of DT. We have successfully generated and purified this immunotoxin from yeast. In addition, we also finished characterizing cytotoxicity toward PD-1-positive cells and MTD (Maximum tolerated dose) in mice of the anti-PD-1-immunotoxin-DT. The cytotoxicity test was performed through MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay. Anti-PD-1-immunotoxin-DT exhibited an EC_{50} of, 1-6 nM against EL4 cells (PD-1-positive cell line) while the EC_{50} against EL4 PD-1-knockout cells was 100 times higher, which indicates the specificity of this immunotoxin against PD-1-positive cells. Moreover, we determined the MTD of the immunotoxin as of 0.5 mg/kg, indicating its strong cytotoxicity. In the future, we will study pharmacokinetic and pharmacodynamic (PK/PD) of the immunotoxin to optimize the dose regimen and then determine the therapeutic effect of the immunotoxin in AD mice models. In summary, through the studies we have found that anti-PD-1-immunotoxin-DT has the therapeutic potential to be developed as a drug candidate in AD diseases.

Antibody-Mediated Depletion of PD-1 (programmed cell death protein 1) positive cells

Yujia Zhai, Shuyun Dong, Mingnan Chen

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The role and function of PD-1 immune checkpoint in cancer and autoimmune disorders has been intensively investigated recent years. Cells that express PD-1 receptor (termed as PD-1-positive cells) are drawing ever-increasing attention. In cancer research, PD-1-positive T cells are employed to suppress tumors; on the other hand, PD-1-positive tumor cells have been reported as initiator of tumors as well as one factor that cause the resistance to PD-1 blockade therapy. In autoimmune diseases, PD-1-positive lymphocytes were found to infiltrate tissues and connected with disease progression. However, there still remains large ambiguity and unknown in the role and function of PD-1 positive cells in cancer and autoimmunity. One of the reasons of such ambiguity is the method chosen by researchers. Up to now, all such researches investigated the role of PD-1 positive cells by either PD-1 blockade or PD-1 gene regulation. They failed to investigate PD-1 positive cells as a whole. To address this issue, we unprecedentedly designed and generated a depleting antibody (D-αPD-1) that is able to deplete PD-1 positive cells as a tool to study PD-1 positive cells. D-αPD-1 has the same variable domain as an anti-PD-1 blocking antibody (RMP1.14 clone) we cloned previously, and its constant domain was designed based on the Fc of IgG2a. D-αPD-1's binding capacities with both PD-1 molecule and FcγRIV, a mouse immuno-activating Fc receptor, were verified. EL4 cells that are PD-1 positive were then transferred into mice followed by single-dose treatment of D-aPD-1 and D-aPD-1's inhibition effect on EL4 tumor initiation was measured. Compared with other groups of mice without D-aPD-1 treatment that have median survival days of approximately 30 days, D-aPD-1 treated group survived the entire study up to 60 days, which indicates the depleting effect of D-aPD-1 on PD-1 positive cells *in vivo*. Furthermore, we also investigated the working mechanism of D-aPD-1 and proved that D-αPD-1 depleted PD-1 positive tumor cells by antibody-dependent cell-mediate phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) mechanism. These results altogether proved the depleting efficacy of D-αPD-1 on PD-1 positive cells. This D-αPD-1 provides a tool for the study on PD-1 positive cells as a whole, and will further benefit the development of related therapy.

Combination of an Oligomeric Sulfated Hyaluronan and Silk-Elastinlike Polymer Protects against Murine Radiation Induced Proctitis

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Radiation induced proctitis (RIP) is a common side effect from radiotherapy treatment of cancers of the lower abdomen or pelvic regions. These cancers, which include ovarian, prostate, and cervical cancers, rank as some of the most prevalent in the world. It is estimated that 5 to 20% of individuals undergoing this type of radiotherapy will develop acute or chronic RIP, experiencing symptoms including abdominal pain, diarrhea, and hematochezia. The combination of these symptoms, primarily driven by inflammation, can lead to cessation of radiotherapy treatment. Current preventative measures are limited primarily to limiting dosage of the fractions of radiation.

Semisynthetic glycosaminoglycan ethers (SAGEs) exhibit a number of anti-inflammatory properties, such as blocking pathogen associated molecular patterns and inhibition of receptors for advanced glycation end products. Administration rectally via an enema shows promise to reduce inflammation in RIP. SAGE however lacks residence time when administered as the sole component in the solution. Silk-elastinlike polymers (SELPs) are thermoresponsive and undergo a sol to gel transition upon heating to physiological temperature. A liquid enema consisting of SELP and SAGE allows for rectal accumulation of SAGE by means of the polymer's gelation following administration.

The radioprotective capabilities of a SAGE-SELP enema was assessed in two cohorts of mice which were irradiated with 37 Gy at the lower abdominal region. These mice were separated into four groups who received prophylactic treatments comprised of PBS, SAGE in PBS, SELP, or SAGE-SELP and one non-irradiated control group. Prior to treatment and sacrifice, lower abdominal pain responses were evaluated, along with animal weights. One cohort's tissues were collected after three days and another at humane endpoints.

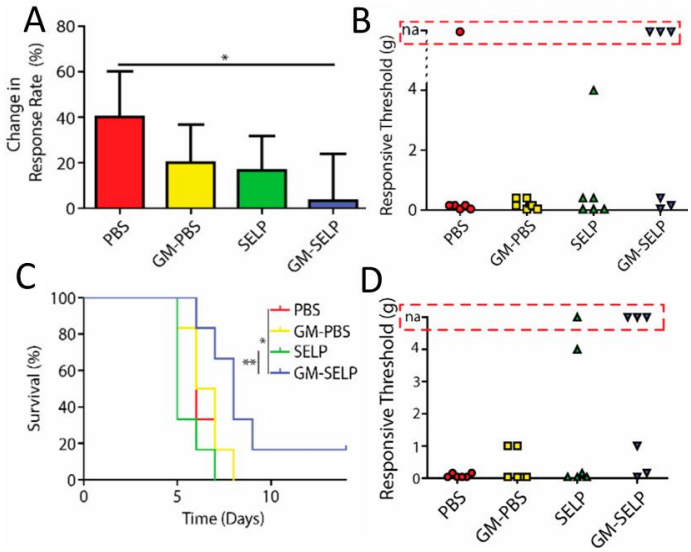

Figure 1: (A) Change in response rates from baseline measurements with the 0.16-g filament in the three-day time points. (B) Threshold required to elicit an allodynic response as measured by an increase in 30% from the baseline. Red box indicates animals with no allodynic response (na). (C) Survival curves of irradiated animals receiving prophylactic treatments. (D) Threshold required to elicit an allodynic response as measured by an increase in 30% from the baseline. Red box indicates animals with no allodynic response (na). (** $p < 0.01$, * $p < 0.05$).

The groups in both cohorts who received either SAGE or SELP or the combination showed increased stimuli thresholds for painful responses, indicating relief from presentation of pain in this model. The three-day mice receiving SAGE-SELP treatment showed significant reduction in response rate to stimuli (Fig. 1a). Of the mice receiving this treatment, 50% did not exhibit allodynic responses after 3 days (Fig. 1b). Within the longer-term cohort, those treated with the SAGE-SELP combination survived 60% longer than other treatment groups (Fig. 1c). Similar allodynic responses were observed in the survival endpoint group (Fig. 1d).

Histology of both cohorts indicated reduced inflammation of the irradiated tissues in those treated with the SAGE-SELP combination, with lower injury scores overall in the 3-day cohort compared to survival timepoints. Presentations of luminal migration of epithelial nuclei and inflammation were most prominent in tissue sections after 3 days. At humane endpoints, histological alterations included a plethora of changes indicating the pathological development of an RIP injury.

This drug polymer combination affects pain in irradiated animals and the development of inflammation in the rectum. This reduction suggests increased health and longevity. When administered prophylactically, the combination shows indication for application in patients for hypo-fractionation radiotherapy. This reduction in both pain and inflammation shows promise for other applications in RIP outside of prophylactic treatments. Alterations to administration and optimization are set to be explored in the future.

Acknowledgement: This research was funded by the National Institutes of Health, grant number 5RO1CA227225.

Feasibility of fluorescent image-guided transoral robotic surgery for HPV+ oropharynx cancers using indocyanine green

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Background: Transoral robotic surgery (TORS) is a minimally invasive approach to treat Oropharyngeal squamous cell carcinoma (OPSCCa). However, delineating tumor margins from normal tissue is still a challenge, as 10-20% of cases have positive surgical margins. Our objective is to investigate the use of indocyanine green (ICG) to demarcate tumor margins in patients undergoing TORS.

Methods: All participants with a diagnosis of HPV+ OPSCCa undergoing TORS were enrolled in a prospective, observational cohort study. Patients were intravenously injected prior to surgery with 25 mg of ICG. During the patients' tumor resection using the Davinci robot, the Firefly ICG filter was used to obtain fluorescent images. Once the tumor and lymph nodes were resected, tissues were imaged using the in-vivo imaging system (IVIS) and subsequently submitted for pathological analysis.

Results: The ICG filter at 780 nm was superior to white light in identifying tumors embedded inside the base of the tongue or tonsils. On post hoc evaluation it was noted positive margins were successfully resected while minimizing the amount of normal tissue resection. A significantly higher concentration of ICG was observed when it was injected 6-8 hrs before surgery compared to 17-18 hrs. IVIS demonstrated an accuracy of 91.3% and a significant correlation (P=0.001) in identifying tumoral tissue in resected samples.

Conclusion: Intraoperative fluorescent guidance using ICG filter during TORS aids in the identification of primary tumors. Intravenous ICG injection 6-8 hours prior to surgery resulted in the highest qualitative concentration of ICG in tumoral tissue.

Funding: The research reported in this abstract was supported by Huntsman Cancer Foundation.

Poloxamer 407 based micellar encapsulation of propofol to reduce its adsorption to the ECMO circuits

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† **Co-first authors**

Background: Extracorporeal membrane oxygenation (ECMO) is a life-saving cardiopulmonary bypass device used in patients with heart and lung failure. Patients on ECMO receive numerous drugs. Dosing is often unknown because the ECMO circuit components can adsorb drugs. The adsorption of drugs by ECMO circuit can be reduced by encapsulation in micelles (Figure 1). We chose propofol because it is widely used in ECMO patients, highly adsorbed and has favorable physicochemical properties for micellar encapsulation.

Methods: The thin-film method was utilized for micelle manufacturing. Hydrodynamic radius, polydispersity (PDI) and zeta potential were determined by Dynamic Light Scattering (DLS). Cryoprotectants, dextrose, sucrose and trehalose were added to the micellar solution to observe the effect of lyophilization on PDI of the formulation after reconstitution. Propofol quantification was determined by HPLC.

Results: The hydrodynamic radius and PDI for drug-free micelles were 21 nm and 0.200, respectively. The zeta potential was -5 mV. An increase in size to 25 nm was observed after incorporation of propofol, but the PDI dramatically decreased (PDI=0.04), indicating lower polydispersity. Studies of lyophilization showed an increased PDI after reconstitution that was reduced by adding cryoprotective agents with dextrose.

Conclusion: We successfully created micelles and loaded them with Propofol. Promising data on size, low polydispersity, and stability were observed. This provides the opportunity to investigate the encapsulation efficiency, drug loading capacity and release kinetics of propofol, followed by *ex-vivo* studies with the ECMO device to compare adsorption between free and micellar propofol.

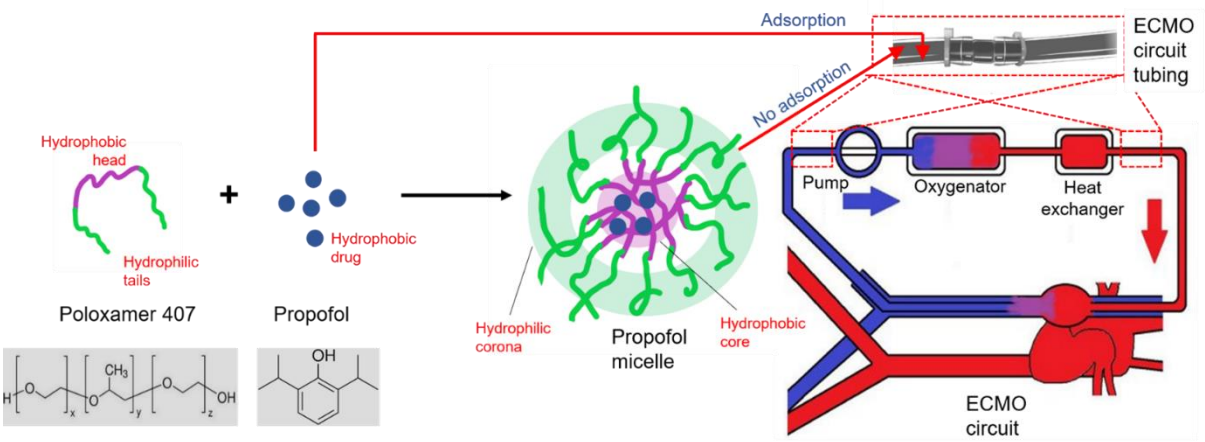

Figure 1. Micellar encapsulation of drugs to prevent ECMO circuit adsorption

Immunotoxicity of Silica Nanoparticles as a Function of Physicochemical Properties

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INTRODUCTION

Silica-based nanoparticles have shown significant potential in biomedical applications such as controlled drug delivery, theranostics, and imaging due to their tunable physicochemical properties. The safety profile of SNPs has not been fully established, which is crucial for clinical translation and advancement. A detailed examination of the interaction of silica nanoparticles with our immune system and the downstream effects is warranted. There is a knowledge gap on how the physiochemical properties of SNPs such as size, charge, geometry and porosity influence their immunotoxicity. A major concern is the saturation of mononuclear phagocytic system. Our objective in this work is to find the saturation doses of SNPs in murine macrophages and investigate their immunotoxicity at these doses and as a function of silica nanoparticle physiochemical properties.

EXPERIMENTAL METHODS

Silica Nanoparticle Fabrication and Characterization: To evaluate the effect of size and porosity on macrophage saturation and immune function, 4 spherical silica nanoparticles were prepared using the modified Stöber process by base-catalyzed hydrolysis and condensation of the silane precursor tetraethyl orthosilicate (TEOS) in water/ethanol mixture in the presence of ammonium hydroxide.[1] Nonporous Stöber silica nanoparticles with a diameter of approximately 50 and 100 nm (SNP50 and SNP100, respectfully) were synthesized as previously described.[2] SNP50 and SNP100 with low polydispersity were obtained through controlling particle size by adjusting TEOS and ammonium hydroxide concentrations. Mesoporous SNPs with a diameter of approximately 100 nm (MSNP100) were synthesized by using the surfactant cetyltrimethylammonium bromide (CTAB) as the structure-directing reagent to obtain a hexagonal pore structure with average pore size of 3 nm. CTAB was removed from the particles' pores via solvent extraction with acidic ethanol solution under reflux. CTAB removal was confirmed via Fourier Transform Infrared Spectroscopy (FTIR). Lastly, hollow mesoporous SNPs with a diameter of approximately 100 nm (HMSNP100) were synthesized as previously described.[2] Particle size, size distribution, and morphology were characterized using transmission electron microscopy (TEM). Hydrodynamic radii and zeta potential were characterized using dynamic light scattering (DLS).

Cell Culture and Viability Assay: RAW 264.7 murine macrophages were cultured according to standard protocol. To determine the maximum tolerated dose (MTD) of SNPs, cells (10,000 per well) were seeded in 96-well plates for 24 h before treatment. Cells were dosed with all four SNPs at different concentrations (800-6.25 μg/mL) and incubated for 24 h. Relative cell viability was

obtained via CCK-8 colorimetric assay. Cells treated with complete medium without particles were used as negative control and cells treated with dimethyl sulfoxide (DMSO) as positive control.

Cell Uptake: To investigate the rate of uptake and saturation doses of silica nanoparticles by RAW 264.7 macrophages, cells were dosed with MTD concentrations of SNPs and particle internalization was determined through quantification of silicon via inductive coupled plasma-mass spectrometry (ICP-MS). Briefly, cells were grown on 12-well plates until confluent and treated with SNPs. At various time points (1, 3, 6, 9, 24, 48, 72 h) the media was collected and cells were washed with PBS and harvested. Cells were lysed using Trizol solution and digested with hydrofluoric acid. ICP-MS was used to quantify SNP internalization.

Annexin/PI assay: Cell death at saturation doses was evaluated by Annexin/PI. Cells were seeded in 12-well plates and grew until confluent. SNPs were added at their saturation dose. After 24 hours incubation, cells were collected and stained with Annexin and PI and analyzed by flow cytometry.

Immune Response Gene Expression Analysis: Cells were incubated with SNPs at their saturation dose and after 24 h total RNA was extracted using Qiagen RNA extraction kit according to the manufacturer's procedure, and a cDNA library was generated using the primers GAPDH, IL4, IL6, IL10, TNFα, IFNγ, CSF1 and CXCL2. Real time PCR was performed and mRNA levels were normalized to GAPDH mRNA levels as reference gene.

RESULTS AND DISCUSSION

The average size, PDI, and saturation doses of all 4 SNPs was established as follows- SNP50: 68 ± 19.3 nm, 0.137, 50 μg/mL; SNP100: 111 ± 27.9 nm, 0.039, 100 μg/mL; MSNP100: 134 ± 47.2 nm, 0.121, 200 μg/mL; HMSNP100: 132 ± 41.3 nm, 0.087, 200 μg/mL. SNP50 had significantly higher uptake, while MSNP100 and HMSNP100 demonstrated higher tolerated doses but lower uptake at saturation doses. Rapid rate of uptake was observed for all 4 particles, with saturation occurring within 24 hours. No apoptosis or necrosis of cells was observed at saturation doses. Gene expression analysis of SNP-saturated macrophages resulted in significant increased expression of IFNγ for all SNPs and an elevated expression of TNFα for the porous particles (MSNP100 and HMSNP100), whereas expression levels of all other genes analyzed were similar to control exhibiting minimal to no immunotoxicity.

CONCLUSION

We conclude that SNP saturation of murine macrophages is dependent on nanoparticle physicochemical properties. Macrophages exhibit minimal toxicity at SNP saturation doses. This gives us the opportunity to further investigate the correlation between the physicochemical properties of SNPs, as a carrier platform, and the mechanisms of immune response alteration *in vitro* and *in vivo*.

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Recombinant Protein-Based Hydrogels for the Development of 3D Bioprinted Bioinks

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Background: 2D cell culture models are used to assess the efficacy of therapeutic compounds during drug discovery and development processes. However, cells behave differently and express proteins at different levels in 2D cultures as compared to complex 3D environments which resemble more closely to their *in vivo* environment. Thus, using 3D models for drug development can lead to more accurate cellular responses when testing compounds, before moving to more expensive animal models. 3D bioprinting is a cutting-edge technology that provides the possibility for spatiotemporal control of the placement of cells, as well as the extracellular matrix (ECM), and biologically active molecules in 3D cultures. 3D bioprinting of cells in biocompatible hydrogels (called bioinks) can provide the structural and molecular cues and help replicate the *in vivo* environment for them. Currently, most 3D printed models use alginate and gelatin-methacryloyl (Gel-MA) as bioinks via extrusion based bioprinting. These bioinks must be crosslinked using a large amount of $CaCl_2$ or be exposed to UV light, which could be detrimental to cell viability.

In this study, we are developing novel recombinant bioinks, based on silk-elastinlike protein polymers (SELPs). SELPs exhibit a thermoresponsive transitioning from a liquid to a solid gel at body temperature without the need for chemical or UV crosslinking. By varying the number and sequence of silk and elastin, we can tune the hydrogel properties such as gelling rate and mechanical strength with molecular precision. Another advantage of SELP is that it is relatively easy to bioengineer allowing for the addition of various motifs to the backbone of the protein polymer, to better represent the ECM.

Methods: We formulated our bioink by testing multiple composite hydrogels comprising SELP, pre-crosslinked alginate and collagen, at different ratios for their printability. We used an extrusion based bioprinter (CELLINK BIO-X) using optimized printing parameters, and selected the best formulation that could be printed into stable 3D structures without the need to further crosslinking. Next, to assess the cytocompatibility of the composite SELP bioink, we incorporated different cell types in the bioink, and printed 3D structures under optimal printing parameters such as speed and extrusion pressure. The structures were cultured under Dulbecco's Modified Eagle Medium (DMEM) in an incubator. The viability was assessed by Live-Dead kit that stains live and dead cells in distinct colors. MCF-7 (breast cancer cell line) and Human Dermal Fibroblast (primary cells) were cultured to confluence, detached and $1x10^6$ cells were suspended in 1ml of SELP bioink. The bioink and cells were loaded to printheads and multiple 3D structures were printed. The structures were submerged in culture media and incubated in cell culture incubators at 37°C and 5% CO_2. The viability was assessed after 24 and 72 hours. We also performed rheological characterization of the optimal SELP bioink by a Malvern Kinexsus Rheometer.

Results: We made formulations comprising pre-crosslinked alginate, collagen and SELP with different ratios. The formulation with 2.75% of alginate pre-crosslinked with 25 mM of $CaCl_2$, 1 mg/ml collagen and 6% SELP had the best printability characteristics. To optimize the printing parameters, we used temperature control printbed that kept the printing area at 37°C, in order to trigger the spontaneous crosslinking of the SELP bioink. The printhead temperature was kept lower to prevent early crosslinking of SELP and keep the viscosity in printable range.

The optimized printing parameters for this bioink were determined as follows:

Temperature of printhead	7 ^{0}C
Temperature of printbed	37 ^{0}C
Extrusion pressure	25 kPa
Extrusion speed	2 mm/s

Other rheological properties of the optimized formulation are the following:

a.

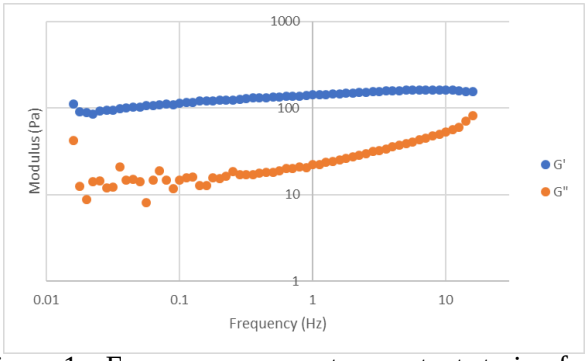

Figure 1a. Frequency sweep at a constant strain of 0.1%.

b.

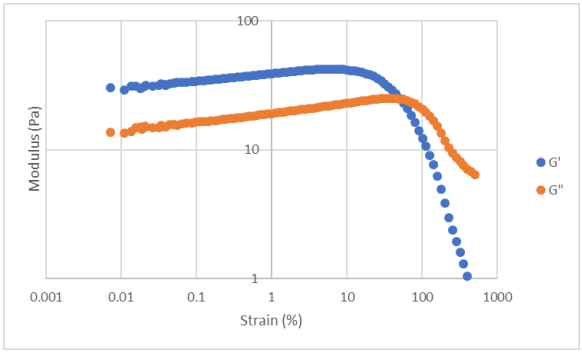

Figure 1b. Amplitude sweep at a constant frequency of 1.59 Hz.

Once the SELP bioink formulation was characterized for printability, its cytocompatibility was investigated by assessing the viability of different cell lines that were printed in SELP bioinks into a 3D structure and cultured under corresponding culture media for different periods of time. The Live-Dead assay revealed a favorable cytocompatibility for our optimized SELP bioink, as the cells remained viable, and no major cell death was observed in structures 3D printed with either MCF-7 cell line or primary HDFs. Our next step in this project is the assessment of cellular behavior and the expression of cellular biomarkers in the 3D bioprinted structures, followed by assessing the drug response in these cultures as compared to 2D cultures.

Funding: This research was supported by the ALSAM Foundation and The University of Utah Intermountain Post-Baccalaureate Research Education Program

Injectable Antibacterial Dressings for the Treatment of Chronic Rhinosinusitis (CRS)

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Chronic rhinosinusitis (CRS) is a chronic health condition that affects the nasal and paranasal sinus cavities. Currently, CRS affects approximately 11.5% of the U.S. adult population and is characterized by epithelial cell death and breakdown of the mucosal barrier. These events lead to opportunistic bacterial invasion, recurrent infections, and biofilm formation, which significantly decrease the outcomes for medical and surgical patients. Treatment options for patients with CRS include systemic and/or topical administration of antibiotics and anti-inflammatory agents. Systemic antibiotics can be effective against planktonic bacteria but are suboptimal in eradicating bacterial biofilms. Continuous use of systemic antibiotics, moreover, is associated with antibiotic resistance. Topical application of corticosteroids and antibiotics have demonstrated limited efficacy in patients due to the currently available delivery systems. Endoscopic sinus surgery is performed after medical management failure; however, bacterial biofilm formation is commonly observed post-operative, reducing the efficacy of surgical intervention. The recent development of intra-sinus corticosteroid-eluting stents has demonstrated improvements in reducing sinonasal inflammation and scarring post-operative but has no effect of preventing biofilm formation. Moreover, the predefined physical shape of the stent limits its application to patients with a permissive anatomical form of the sinus.

The objective of this investigation was to develop a drug delivery system that conforms to the sinonasal cavity and provides the controlled release of silver nanoparticles (AgNp) and hyaluronic acid (HA) for the treatment of CRS. This therapeutic strategy aims to prevent biofilm formation without the risk of developing antibiotic resistance with the use of anti-bacterial AgNps, while also reducing sinonasal inflammation with anti-inflammatory HA. Dosing optimization studies for each component were first performed, determining that the optimal concentration of AgNp against *Pseudomonas* (*P.*) *aeruginosa* and *Staphylococcus* (*S.*) *aureus* proliferation was 32 µg/mL and 125 µg/mL, respectively. Similarly, 2 mg/mL HA inhibited the release of IL-6 and IL-8 release from human macrophages upon stimulation with bacterial LPS. Further investigations with 125, 625, and 1250 µg/mL AgNp against these *P. aeruginosa* and *S. aureus* in a crystal violet assay resulted in the inhibition of biofilm for all concentrations. To increase the residence time in the sinus cavity and sustained therapeutic activities of AgNp and HA, an *in-situ* gelling delivery matrix was fabricated using silk-elastinlike protein polymer (SELP) combined with the optimal determined doses of AgNp and HA. Incorporation of AgNp and HA into SELP hydrogels allows for the formulation to be liquid at room temperature, enabling easy administration to patients via a catheter. Due to the properties of SELP, the formulation then gels at physiological temperature, enabling the sustained release of AgNp and HA. Rheological measurements showed that the

formulation can be easily applied through a catheter and that incorporation of AgNp and HA does not abrogate the temperature responsive *in-situ* gelling property of the SELP matrix. The anti-bacterial effects of the SELP, AgNp, and HA formulation were assessed in a radial diffusion assay against *P. aeruginosa* and *S. aureus*. A distinct zone of inhibition was observed for both strains tested. Herein, we have developed and characterized the *in-situ* gelling and *in-vitro* anti-bacterial effects of a new formulation comprised of SELP, AgNp, and HA for the prevention of biofilm formation in patients with CRS.

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Targeting breast tumor-associated ECM using water soluble copolymer conjugated with collagen hybridizing peptides

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Cancer progression is accompanied by changes in the surrounding tumor-associated extracellular matrix (ECM), which can be targeted to improve tumor drug delivery. The extracellular matrix is dynamically involved in many aspects of cell growth and survival and plays an active role in cancer etiology. Tumor ECM, compared to healthy ECM, exhibits increased collagen deposition and remodeling, due to upregulation of ECM-degrading enzymes. The collagen surrounding tumors is remodeled at a faster rate resulting in a higher proportion of monomeric collagen strands compared to healthy tissue. We capitalize on this distinguishing feature, along with the enhanced vascular permeability associated with tumors, to increase tumor localization of polymer carriers. We present water soluble copolymers targeted to denatured collagen. We synthesized *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers with side chains terminated in collagen hybridizing peptides (CHPs), and we evaluated their ability to bind to denatured collagen and to localize in an orthotopic MDA-MB-231 murine breast cancer model (n=5) as well as their biodistribution. Our results indicate that the targeted copolymer-peptide conjugates exhibit increased tumor localization compared to non-targeted copolymers with scrambled (control) peptides, as well as an increased tumor retention compared to CHPs without polymer backbone. Nanocarriers targeting collagen can enable opportunities to deliver drugs that act therapeutically on the ECM. This system can serve as a vehicle to facilitate the translation of promising ECM-acting drugs in a localized fashion where their efficacy is improved and toxicity reduced.

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Transcellular pathways of bile acid conjugated solid nanoparticle in Caco-2 cells

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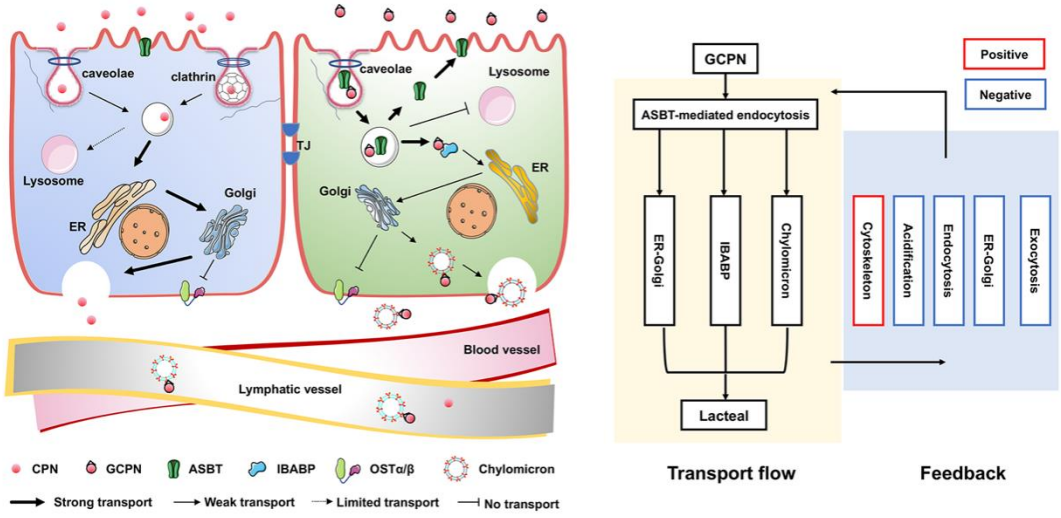
Bile acid transporter-mediated oral nanomedicines delivery has been a promising strategy to overcome the poor oral bioavailability of BCS class III/IV drugs and biologics. In human body, bile acids are synthesized in the liver and stored in the gallbladder. At meals, bile acids are secreted into the duodenum and emulsify the dietary fat. In the ileum, 95% of bile acids are reabsorbed into the portal vein and transported to the liver, with only ~5% excreted in feces. This process is called "enterohepatic recycling". The high recycling ratio of bile acids is profited from their transporters: apical sodium dependent bile acid transporter (ASBT), which takes bile acids from intestinal lumen to epithelial cells; ileal bile acid binding protein (IBABP), which transports bile acids from apical side to the basolateral side; organic solute transporter α/β (OSTα/β), which assists the exocytosis across the basolateral membrane. Targeting these transporters, especially ASBT, has been proven to be effective in enhancing the transport of bile acid-modified nanomedicine across the enterocytes.

Despite the success in oral administration of bile acid-modified nanomedicines in preclinical studies, the mechanisms of their transport are still illusive. Since the binding cavity size of ASBT is narrow (6Å×12Å×14Å), the bile acid-modified nanomedicines are unlikely to enter the enterocytes simply by transporters' pumping function. Meanwhile, although bile acids help emulsification of fats in small intestine, the digested fats and the bile acids are absorbed separately: the bile acids are absorbed in the ileum via ASBT, while the fats are mainly taken up in the duodenum. In the enterocytes, the digested fats are packed up into chylomicrons and transported into the mesenteric lymphatic vessels. With conjugation of bile acids on the surface, the bile acid-modified nanoparticles might transport via the ASBT pathway, chylomicron pathway, or an entirely new route.

To clarify the transport pathways of bile acid-modified nanoparticles, we synthesized glycocholic acid-conjugated polystyrene nanoparticles (GCPN), and studied their transport behaviors in Caco-2 cells. The results showed that GCPN demonstrated a 4-6-fold uptake compared to that of non-conjugated nanoparticles. The pharmacological inhibition and gene knock-down studies demonstrated that both GCPN and the control nanoparticles shared caveolae-mediated pathway and were internalized with the assistance of actin and dynamin, but GCPN also transported via Arf6 and not through clathrin-mediated pathway. The colocalization and separation between ASBT and GCPN were observed, suggesting the ASBT-mediated endocytosis and ASBT recycling properties. The colocalization with IBABP and the inhibition study of OSTα/β demonstrated that IBABP but not OSTα/β participated in the GCPN transport. The endoplasmic reticulum to Golgi (ER-Golgi) pathway was validated by colocalization study and ER-Golgi inhibition study, while few GCPN were observed in lysosomes. The induction effect of chylomicron study was tested by ELISA confocal microscopy, which exhibited that both GCPN and glycocholic acid could induce the formation of chylomicron, while the control group not. The

isolation of secreted chylomicron validated their association with GCPN, indicating that GCPN but not the control particles were transported via the chylomicron pathway.

Compared with endocytosis, the difference between the transcytosis of GCPN and the control nanoparticles was only 2-3 folds. The discrepancy between endocytosis and transcytosis was tested by genomics, with more than 24000 genes identified and the Gene Oncology was performed to analyze the pathways of biological process (BP), molecular function (MF) and cellular component



(CC). Compared with the control nanoparticles, the majority of the transport-related pathways were downregulated when treated with GCPN, suggesting that the Caco-2 cells responded to the transport flow of GCPN in a negative feedback pathway. To verify this, exocytosis of GCPN and the uptake of courmarin-6 loaded micelles in GCPN-treated cells were further studied. The result showed that the transport was weakened after GCPN treatment, which was consistent with the genomics study result. This study will set up a stepping stone for design of bile acid-modified nanomedicines and regulation of their transport.

Heteroreceptor Crosslinking Induces a Synergistic Therapeutic Response in Malignant B Cells

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Drug-Free Macromolecular Therapeutics (DFMT) is a new paradigm in macromolecular therapeutics that induces apoptosis in target cells by crosslinking of receptors without the need for low molecular weight drugs.[1,2] Programmed cell death is initiated via a biomimetic receptor crosslinking strategy using a two-step approach: i) recognition of cell surface antigen by a morpholino oligonucleotide-modified antibody Fab' fragment (Fab'-MORF1), ii) followed by crosslinking with a multivalent effector, human serum albumin grafted with multiple complementary morpholino oligonucleotides (HSA-$(MORF2)_x$). As a platform technology, we have demonstrated this approach effective in crosslinking of CD20 and CD38, respectively. For example, crosslinking of CD20 receptors-initiated apoptosis not only in vitro,[1,3] in vivo,[4] but also on cells from patients diagnosed with various B cell malignancies.[5]

Herein we show that simultaneously engaging and subsequently crosslinking both of these targets ("heteroreceptor crosslinking") can further enhance the apoptosis induction capacity of this system. To accomplish this, we simultaneously incubated Raji ($CD20^+CD38^+$) cells with anti-CD20 and anti-CD38 Fab'-MORF1 conjugates, followed by addition of the macromolecular crosslinker, HSA-$(MORF2)_x$, to co-cluster the bound receptors. Fab' fragments from Rituximab and Obinutuzumab were employed in the synthesis of anti-CD20 bispecific engagers (Fab'$_{RTX}$-MORF1 and Fab'$_{OBN}$-MORF1), whereas Fab' fragments from Daratumumab and Isatuximab (Fab'$_{DARA}$-MORF1 and Fab'$_{ISA}$-MORF1) targeted CD38.

All heteroreceptor crosslinking DFMT combinations demonstrated potent apoptosis induction and exhibited synergistic effects as determined by Chou-Talalay combination index[6] studies. *In vitro* fluorescence resonance energy transfer (FRET) experiments confirmed the co-clustering of the two receptors on the cell surface in response to the combination treatment. The synergistic therapeutic effect was further explored by evaluating the effect of the combination on key apoptosis signaling events such as mitochondrial depolarization, caspase activation, and lysosomal enlargement. Finally, a xenograft mouse model of CD20+/CD38+ non-Hodgkin's lymphoma was employed to demonstrate the enhanced efficacy of the heteroreceptor-crosslinking DFMT *in vivo* versus single-target systems.

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A New Platform Technology of Antibody-Polymer-Drug Conjugates with Potential Treatment of Hematologic Malignancies and Solid Tumors

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Cancer is one of the leading causes of death in the world. Targeted therapy is a treatment option involving the administration of drugs conjugated to antibodies that are selective for cancer cells leaving healthy cells relatively unharmed. However, conventional antibody-drug conjugate technology for cancer is associated with significant adverse effects due to the use of highly toxic toxin payloads. Alternative approaches are needed to optimize the design of antibody-drug complexes for targeted disease therapy[1-4].

Aiming to combine high efficacy and enhanced tolerability, we recently developed a new therapeutic strategy consisting of an antibody and semitelechelic *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-drug conjugate (*ST*-P-drug). The latter was selectively attached to antibody via reduced disulfide bonds at the hinge region[1,2]. Using this novel strategy with rituximab (RTX) to target CD20, we synthesized an antibody drug conjugate containing the HPMA polymer-epirubicin precursor (*ST*-P-EPI). Of note, the drug-to-antibody ratio (DAR) exceeded 40 without interfering with solubility or pharmacokinetics. RTX-P-EPI was highly effective in both RTX-sensitive and RTX-resistant mouse models of non-Hodgkin lymphoma (NHL)[2].

Following the initial success of RTX-P-EPI, we expanded this novel strategy to several antibodies that are FDA approved for clinical use, such as trastuzumab (Herceptin) and daratumumab (DARA), to demonstrate the therapeutic potential of the new constructs toward hematologic malignancies and solid tumors. Moreover, we substantially modified the synthetic approach. We synthesized an azide-containing semitelechelic polymer-drug precursor in one step. This novel strategy allows for a wide variety of drug payloads to be readily conjugated to a clickable antibody with high drug loading while maintaining excellent physicochemical properties (Fig. 1A & B).

Two examples will be introduced. The first is DARA-P-GDC 0980 (SY120418), consisting of an FDA approved anti-CD38 monoclonal antibody conjugated with multiple GDC-0980, a dual inhibitor of the PI3K/mTOR pathway, via an HPMA polymer carrier for the treatment of multiple myeloma (Fig. 1C). The specific binding and cellular uptake of anti-CD38 ADC were confirmed with $CD38^+$(RPMI 8226) and $CD38^-$(U266) cells and tumor-bearing mice. The second example is the validation of RTX-P-EPI on NHL in the presence of natural killer (NK) cells, to create a more clinically relevant environment. Further work on these agents is ongoing. We anticipate this new design of ADC to have the synergistic potential of immunotherapy and macromolecular therapeutics while lowering the risk of toxicity.

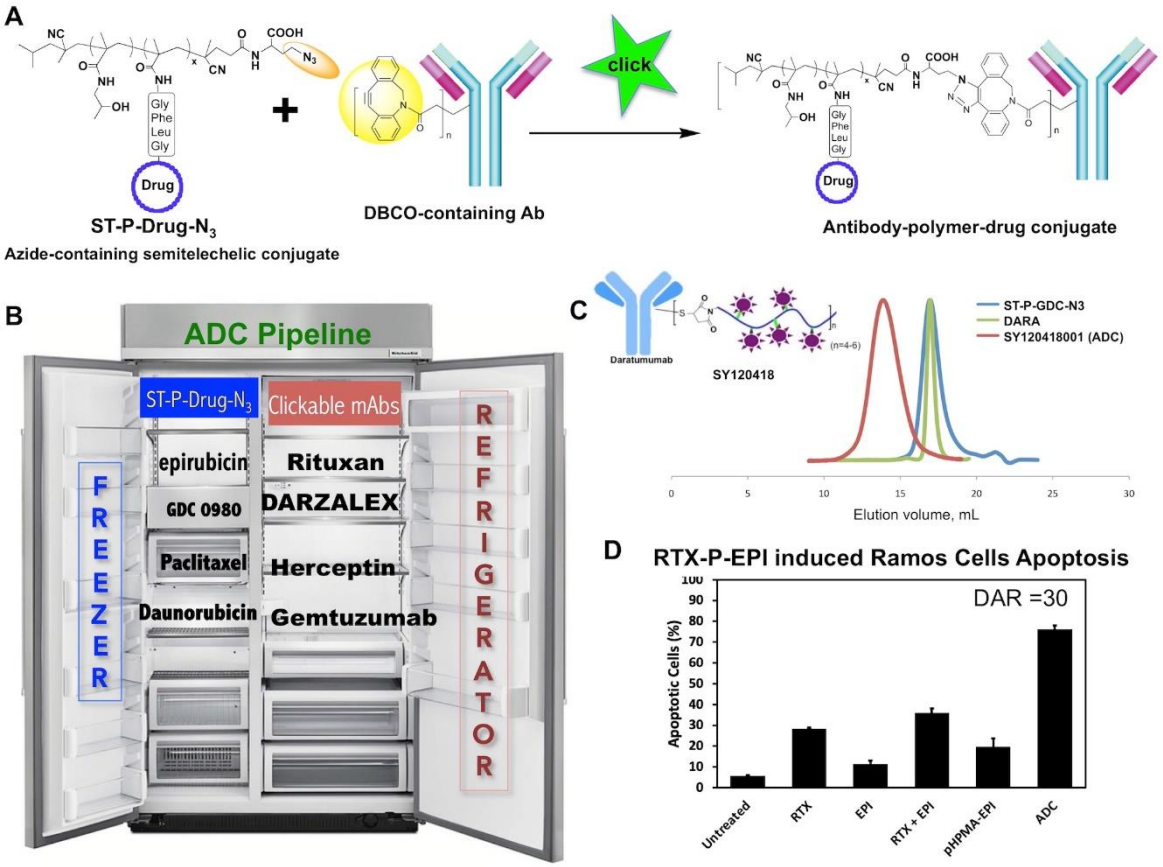

Figure 1. Schematic illustration of newly developed synthetic approaches via click chemistry (A**),** and the innovative platform technology with versatile constructs (B). Size-exclusion chromatography showed DARA-P-GDC with high drug-to-antibody ratio (DAR) and good homogeneity (C); Enhanced apoptosis level strongly suggests highly effective RTX-P-EPI as a next generation therapeutic strategy (D).

Acknowledgement. The research was supported in part by NIH grant RO1 CA246716 from the National Cancer Institute (to JK), Department of Defense grant W81XWH-20-1-0573 (to JY), Huntsman Cancer Institute ET grant 39024 (to DS/JY) and University of Utah Ascender Grant funding 51900528 (to JY).

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Optimized Immunotherapy for Cancer Treatment: Polymer-Enhanced Combination of Immunogenic Chemotherapy and PD-L1 Degradation

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Immune checkpoint blockade (ICB) using antibody inhibitors (anti-PD-1 or anti-PD-L1 mAbs), enables pre-existing tumor-specific T cells to kill cancer cells. Although ICB has demonstrated significant clinical benefits in some malignancies, low response rates in most cancers including breast cancer, and acquired resistance are still major challenges.

We designed and synthesized a multivalent polymer-peptide antagonist to PD-L1, denoted MPPA, which utilizes receptor crosslinking as a molecular switch. MPPA, as an alternate checkpoint inhibitor, not only blocks the interaction of PD-1/PD-L1, but also crosslinks multiple PD-L1 receptors at the cell surface, which mediates endocytosis of MPPA crosslinked PD-L1 complexes, and shunts PD-L1 from recycling to lysosomal degradation. These combined actions provide persistent PD-L1 suppression, leading to enhanced T cell mediated cytotoxicity. In particular, when MPPA is paired with KT-1, a backbone-degradable *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-epirubicin conjugate, KT-1 effectively elicits immunogenic cell death to "heat up" immunologically cold tumors, resulting in a sensitive microenvironmment to MPPA. Our in vitro and in vivo data in 4T-1 murine triple negative breast cancer (TNBC) model, colon cancer (CT26) model, metastatic Lewis lung cancer (LLC-1) models, and MMTV-PyMT transgenic mouse model demonstrate that MPPA, as an innovative, two pronged PD-L1-targeted therapy, in combination with KT-1, has higher efficacy, as well as lower non-specific toxicities compared with current clinical treatment regimens[1,2].

Recently, we focused on MPPA in combination with KT-1 for treatment of FVB mice bearing large MMTV-PyMT tumors (~500 mm^3), which recapitulates clinical advanced stage of TNBC. The immunosuppressive tumor microenviroment, for example, lack of CD8+ T cell infiltration, high level of MDSCs (CD11b+Grl+) and Treg (CD3+CD4+Foxp+) was significantly remodeled after KT-1 treatment. We will present our recent studies evaluating the effects of tumor-associated macrophages. We believe a better understanding of how advanced tumors respond to the combination treatment of ICD-inducing and PD-L1 blocking polymer conjugates is critical to the rational design of cancer immunotherapy. Acceptance of the combination therapy of KT-1 and MPPA by the Nanotechnology Characterization Laboratory for preclinical evaluation will certainly enhance the speed of translation of the new approach into the clinic[3].

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Cancer immunotherapy with ROS producible hMSC-based modulator

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Introduction

Activating immune system via immune cell and pro-inflammatory cytokine stimulates antitumor response and tumor growth inhibition. To control autoimmunity and immune-based disease resistance, multipurpose carriers have been developed and used for several clinical trials. However, due to short half-life of cytokines, the importance of delivering cytokines to target disease sites for extended periods is increasing.

To resolve the limitations, we developed reactive oxygen species (ROS) producible human mesenchymal stem cell (hMSC) as an immune modulator. The hMSC has proved potential as a cancer therapy because it is able to produce various cytokines and it has homing effect to metastasis sites. We conjugated polymers and photosensitizers (PS) on the cell surface (hM-PP) via copper free click chemistry. Once the hM-PP migrated to the inflammation sites, laser irradiated hMSC released pro-inflammatory cytokines which could be used as immune triggering agents. Therefore, hM-PP could be a therapeutic platform for local immune regulator.

Experimental

We generated azide groups on the cell surface and labeled with hydrophilic polymer and photosensitizer (PP). The synthesis of polymer and photosensitizer (PP) was confirmed by ^{1}H-NMR and efficiency of labeling was validated by flow cytometry and confocal microscopy. Photodynamic mediated cytotoxicity of hM-PP was measured under various laser powers.

To prove that hM-PP has a potential for cancer immunotherapy, we designed K1735 and SK-BR-3 tumor bearing mouse model. The cancer specific migration and surface conjugation of hM-PP was demonstrated by NIR fluorescence imaging and immunohistochemistry imaging of tumor. The solid tumor immunotherapeutic efficacy of hM-PP was confirmed by measuring tumor volume, TUNEL and H&E staining of tumor.

Results and Discussion

We have demonstrated ROS producible hMSC-based immunomodulators of which tumor target efficacy and photo-reactivity. The hM-PP was fabricated by labeling stem cell surface with hydrophilic polymer and photosensitizer (PP) through copper free click chemistry. The laser irradiated hM-PPs specifically migrated to metastasis sites and secreted pro-inflammatory cytokines such as IL-8, IL-6, and heat shock protein 70. The secreted cytokines produced new immune factors which induce maturation of immune cells such as B cells, T cells, antigen presenting cells, and natural killer cells. hM-PP overcame many conventional limitations of immunotherapy by avoiding systemic toxicity. These results supported that laser irradiated hM-PP could regulate the immune system at specific tumor sites.

Consequently, we have demonstrated through various experiments that hM-PP can be a potential platform for local immunomodulator.

Photoactivatable nanocomposite for nasal vaccine delivery

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Introduction

Nasal vaccines have great potential to induce successful immunization with their safety and convenience. The nasal passage is the primary site of most infectious pathogens. For the reason, mucosal immunity in nasal has a great potential to induce simultaneous cellular and humoral immune responses through stimulation of nasal-associated lymphoid tissues. In addition, nasal administration has low risk of bloodborne infection, compared with parenteral administration. However, conventional nasal vaccines have a low antigen delivery rate due to their low affinity to the mucosal surface, rapid removal, and highly viscoelastic mucus layer called mucus barrier. To successfully deliver antigen to antigen presenting cells or tissues, nasal vaccines are required for penetration of mucus barrier.

Experimental

We designed a photoactivatable vaccine (PhotoVac) with photochemical immunomodulatory functions that exhibited efficient nasal antigen delivery and antigen-specific immunity against pathogenic viruses. To photoactivate a antigenicity of PhotoVac, we used a photosensitizer conjugated with cationic poly(amino acid) through amide bonds. The PhotoVac was readily prepared via electrostatic self-assembly of high molecular weight antigens such as hemagglutinin (HA).

To estimate the photoactivity of PhotoVac, FITC-labeled PhotoVac was intranasally administrated to mice (BALB/c, male, 6 weeks, n=6), and 671 nm wavelength laser was irradiated. The intranasal residence time of PhotoVac was verified using confocal laser scanning microscopy. To confirm systemic immunization of mouse, immunization factors (*i.e.* $CD4^+$ and $CD8^+$ T cells, antigen specific antibodies, and IFN-γ) were analyzed with flow cytometry and confocal images. The therapeutic effects of PhotoVacs were estimated by body weight and survival rate of mice.

Results and Discussion

The PhotoVac consisted of photosensitizer conjugated cationic poly(amino acid) and viral antigen such as HA. The cationic poly(amino acid) increased mucoadhesive property and retention time through their carboxylic acid groups. In addition, reactive oxygen species (ROS) generated by photosensitizer enhanced penetration of mucus barrier and stimulated immune responses. ROS also improved tissue penetration and photochemical internalization into cellular barriers. With these mechanisms, PhotoVac successfully penetrated the mucosal barrier and induced antigen specific immune responses via laser irradiation. In *in vivo* study, PhotoVac treated mice with laser irradiation were 100% survived and 8.5% weight loss at 2 weeks, which demonstrated efficiency of PhotoVac compared with free HA treated group survived only 40%. In results, combination of PhotoVac and laser irradiation has verified an effective vaccine against infectious virus. Moreover, PhotoVac provide an alternative strategy as a new type of vaccine platforms.

The alternative antibacterial treatment with *Helicobacter pylori*-selective agent

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Introduction

Helicobacter pylori (*H.pylori*) is known as one of the main factors that induce gastric inflammatory diseases and gastric adenocarcinoma. For these reasons, triple therapy using proton pump inhibitors, clarithromycin, and imidazole or amoxicillin have been developed to treat *H.pylori*. However, medical problems of triple therapy such as inducing antibiotic resistance, removing the unpredictable metabolic bacteria were revealed recently. Thus, it is essential to develop the advanced *H.pylori* treatment without inducing antibiotic resistance and side effects.

Photodynamic therapy (PDT) with photosensitizer (PS) that produces reactive oxygen species (ROS) under laser irradiation has been drawn enormous interest since their high therapeutic efficacy to kill the bacteria. In addition, PDT is able to continuous treatment avoiding antibiotic resistance which is one of the major problems of conventional *H.pylori* triple therapy, PDT has an advantage. However, there are remaining difficulties that non-targeted PDT therapy brings undesirable phototoxicity to normal cells. Therefore, in order to use the advantages of PDT therapy, fabricating the *H.pylori*-targeted photosensitizer is indispensable.

Experimental

In this study, we designed an *H.pylori*-targetable photodynamic agent (HTPA) based on the oligosaccharide that recognizes the SabA on the outer membrane of *H.pylori*. The *H.pylori*-targetable oligosaccharide and ROS producible hydrophobic PS were conjugated to the amine-containing polypeptide. To characterize the properties of HTPA, zeta-potential, water solubility, and photoactivity were measured. The human gastric adenocarcinoma cells were used for evaluating the cytotoxicity and phototoxicity of HTPA. Live/dead assay was conducted to confirm the interaction between HTPA and *H.pylori* using CLSM. After HTPA was administered orally in *H.pylori* infected C57BL/6 mice, laser irradiated using a fiber-coupled laser system. *H.pylori* therapeutic effects and gastric pathological analysis were conducted with Colony-Forming Unit assay, body weight change, H&E, and immunohistochemistry.

Results and Discussion

HTPA was manufactured by conjugating polypeptides with oligosaccharides and hydrophobic photosensitizer. Through *in vitro* tests, we validated minimal bactericidal concentration to evade non-specific cell cytotoxicity. In addition, the targeting abilities and antibacterial effects of HTPA were verified using a bacterial live/dead assay. Afterward orally administrated HTPA recognize and interact with the SabA in *H.pylori*-infected mice model, it caused biological molecule damages through ROS generation under the laser irradiation. In *in vivo* experiments, HTPA showed an efficiently antibacterial PDT effects with avoiding antibiotics resistance and side effects of the existing therapies.

HTPA was fabricated to replace the conventional *H.pylori* treatment that has problems such as inducing antibiotics resistance strains and low efficiency. Through various experiments, HTPA proved its antibacterial and targeting abilities. As a result, HTPA gives possibilities as one of the optimal treatments for *H.pylori* -induced diseases. Furthermore, HTPA showed their potentials that are able to pioneer the new type of treatment that is differentiated from the existing *H.pylori* treatment.

Photo-sensitive multivalent polymer for inhibiting virus infection

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Introduction

Influenza viruses are the most common causes of human respiratory infections. Seasonal influenza pandemics spread a significant portion of the world's population. However, using the antiviral agents to the influenza pandemic is less effective because of viral mutation and virus resistance to antiviral drugs. Therefore, it is necessary to develop that prevents the virus from interacting with the host cell rather than reduce the activity of the virus itself.

Influenza virus is an enveloped virus made up of glycoproteins like hemagglutinin and neuraminidase. Viral infection is initiated by the release of viral genetic materials through the binding of hemagglutinin with specific sugar of glycoproteins on the cell surface. Through the mimicking the elements of adhesion site on host cell, it can prevent viral infection.

Experimental

Herein, we designed photo-sensitive multivalent ligand polymer (PSM) to inhibit the virus from interaction with the host cell. Multivalent ligand binds to the virus with high affinity and prevents virus binding to host cells. Since Multivalent ligand structure blocks interaction with host cells but does not eliminate virus activity, infection cannot be completely prevented. To compensate for these limitations, we conjugated photosensitizer to multivalent ligand polymer to collapse the viral membranes.

To verify the interaction with influenza virus proteins through multivalent structure, saturation transfer difference (STD)-NMR were used to compare PSM and monovalent structure. The interaction between influenza virus and PSM was confirmed by immunofluorescence and transmission electron microscope (TEM) imaging. With 670nm lase irradiation, PSM generated reactive oxygen species due to photosensitizers. Antiviral effect of PSM showed through plaque assay with MDCK cell line. In BALB/c (SPF) mice were infected intranasally with influenza H1N1 virus, and PSM also treated via intranasal. Their therapeutic effect was demonstrated with virus titer in lung and body weight recovery.

Results and Discussion

To synthesize multivalent structure, natural polymer and glycoprotein was conjugated through reductive amination reaction. Then the photosensitizer was linked to inactivate the influenza viruses. Under laser irradiation, PSM generated reactive oxygen species and damage to viral membrane. From TEM images, it was confirmed that the PSM effectively binds to the virus through multivalent ligand and destruct the viral membrane under laser because of lipid oxidation. When the expression level of hemagglutinin according to the laser was confirmed through western blot, it was observed that the protein of hemagglutinin decreased as the amount of PSM increased. Also, plaque reduction assay illuminated that the virus was disrupted by the photosensitizer and exhibited an antiviral effect after laser irradiation. In mice infected with influenza virus, the group of treated PSM showed weight recovery demonstrating antiviral effect. And when the survival rate for 14 days was confirmed, only the group of treated PSM with laser showed 100% survival rate.

Consequently, PSM showed stronger interaction with influenza virus than monovalent structure and induced inactivation of the viruses with laser.

Influenza pandemic has a problem that a new influenza virus caused by viral mutation has little or no existing immunity in human. Virus inactivation ability of PSM has the potential to be demonstrated even in mutated viruses. Therefore, PSM has therapeutic efficacy in various types of influenza viruses.

Gastrointestinal Cancer Therapy Using Targeted Photosensitizer

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Introduction

Photodynamic therapy (PDT) is a treatment method that selectively kills tumor cells using a photosensitizer (PS) for the generation of reactive oxygen species (ROS) by the specific wavelength laser irradiation. Gastrointestinal (GI) cancer, one of the most frequent cancers in modern people, can induced the entire digestive system such as colorectum, stomach, and liver. With the advancement of medical technology, the 5-year survival rate is increased, but there are still patients who are suffering from GI cancer.

There are many treatments that are being operated for GI cancer therapy. Among them, endo-laparoscopic surgery has been the preferred process in the treatment of GI diseases due to less invasive treatment and effective cancer removal. However, there are concerns about recurrence by tumor cells that remain after surgery. To solve these problems, diverse studies are being conducted. The purpose of this study is to kill tumor cells using the receptor overexpressed in GI tract tumors.

Experimental

In this study, we combined a GI tract cancer targeting molecule with a photosensitizer that can generate ROS when irradiated with specific wavelength light. Also, PS combined hydrophilic polymer to improve water insolubility. Thus, the final formulation (targeted hydrophilic photosensitizer, THP), was confirmed synthesis using UV–vis spectroscopy, 2% agarose gel electrophoresis, and ^{1}H-NMR. And we observed ROS with singlet oxygen sensor green (SOSG) when irradiated with a specific wavelength laser on PS.

Furthermore, we observed *in vitro* phototoxicity of THP in various cell lines. It was confirmed through Live&Dead assay that cells were killed only in the laser-irradiated area. This showed the effects of PDT to reduce non-specific systemic toxicity. In addition, the cellular uptake in normal cells and cancer cells was compared and confirmed by confocal laser scanning microscopy (CLSM). At last, to confirm the role of THP as a fluorescence dye, *ex vivo* chromoendoscopic examination was operated.

Results and Discussion

Through these experiments, the synthesis yield of THP was observed about 90% or more. And ROS generation of THP was 1.67-fold higher than HP when laser irradiation. It means that self-assemble of THP inhibited by the targeting molecule which prevented self-quenching of THP. *In vitro* experiments, we observed the specific targeting ability of THP through the result which cells with high expression of the receptor showed higher fluorescence.

Thus, THP has an advantage of being able to visually detect the diseased area according to the color of PS (*i.e.*, The green color photosensitizer is well visible in contrast to the red color of the

living tissue). Also, it has advantage of being a system that can be easily sprayed after endo-laparoscopic surgery. THP has a strong point that the non-specific toxicity, which is a disadvantage of existing antitumor drugs, and can be overcome through PDT and targeting molecule production. In addition, it can be used for other cancers or diseases by replacing the targeting molecule, in that it is expected to serve as a new drug delivery platform.

Cancer immunotherapy using photosensitizer loaded carbon dot

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Introduction

The photodynamic therapy (PDT), photo-responsive therapeutic method, could eliminate tumor. The photosensitizer generates reactive oxygen species and singlet oxygen to induce cancer cell death when laser irradiated with the specific wavelength. That induces apoptotic or necrotic cancer cell death and releases damage-associated molecular patterns (DAMP). Therefore, PDT could induce immunogenic cell death and lead to immunogenicity at tumor environment.

However, the photosensitizer is difficult to use in PDT due to their hydrophobic and low targeting efficiency. To overcome these limitations, we developed a pH sensitive carbon dot (PD). The carbon dot has lots of advantages, such as low toxicity, good water solubility, high emission quantum yield, photostability, and biocompatibility. In this study, pH sensitive property was introduced at carbon dot to target tumor site and load photosensitizer into carbon dot (p-PD).

Experimental

The PDs were synthesized using microwave irradiation for 3 min. The properties of PDs were confirmed by FT-IR spectroscope, dynamic light scattering, UV−Visible spectrophotometer, fluorescence spectroscopy, and transmission electron microscopy. The behavior of p-PDs with pH change was evaluated using UV-vis spectrophotometer, size change, and flow cytometry.

Results and Discussion

The PDs showed general properties of carbon dot that bright luminescence under UV irradiation, small size (below 10 nm), and good water solubility. When the pH was changed from 7.4 to 6.5, surface charge of PDs changed from negative to positive. The photosensitizer was loaded into PDs via hydrophobic interactions and maintained properties of photosensitizer and PD. Compared with p-PDs and free photosensitizer, p-PDs enhanced ability to generate singlet oxygen. When the pH was changed from 7.4 to 6.5, singlet oxygen generating ability of p-PDs further increased.

The intracellular uptake of free photosensitizer and p-PDs at pH 7.4 and 6.5 was investigated using flow cytometry. The photosensitizer fluorescence intensity was similar in the free photosensitizer treated group at the change of pH from 7.4 to 6.5, whereas increased in the p-PD treated group. The photodynamic therapeutic effect of p-PDs was investigated via cell viability test. Under laser irradiation, the p-PDs treated group decreased cell viability compared to the free photosensitizer treated group. However, the p-PDs treated group exhibited negligible cytotoxicity without laser irradiation. Furthermore, pro-inflammatory response was evaluated to demonstrate immunogenic cell death (ICD) of p-PDs. Without laser irradiation, calreticulin positive population was low in all groups. However, calreticulin positive population increased when laser irradiation in photosensitizer and p-PDs treated groups. More importantly, p-PDs showed the highest calreticulin positive population at pH 6.5 upon laser irradiation. DC maturation was performed by exposure to DAMPs, released antigen, and DC maturation effect using anti-CD80 and anti-CD86 expression level. The p-PDs treated group with laser irradiation at pH 6.5 included the highest CD80 and CD86 expression DC.

The p-PDs were injected into tumor baring mouse via intravenously administration and accumulated in tumor tissue by EPR effect. The fluorescence of p-PDs in tumor tissue increased rapidly 1-2 h post injection and remained until 24 h. However, the fluorescence of free photosensitizer was not as high as that of p-PDs and remained only 4 h. To demonstrate that the PDT triggered ICD of tumor cell, we established bilateral tumor baring mice model. Mice were treated with PBS, PDs, free photosensitizer or p-PDs, and only the left tumor site was laser irradiation. The left tumor growths of laser irradiated p-PDs treated group were inhibited. Importantly, the right tumor growths of laser irradiated p-PDs treated group were hampered via photo-induced antitumoral immune response. Furthermore, the population of T, NK, and DC cells in tumor of laser irradiated p-PDs treatment group was higher than other groups.

Antigen and adjuvant co-loaded liposomal nanoparticles for cancer immunotherapy

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Introduction

The lymphatic system is a network of lymphatic vessels and lymph nodes (LNs) that circulate immune cells and provide sites for antigen presentation and immune activation [1]. Antigen presentation of antigen-presenting cells (APCs) to resident lymphocytes of the LN modulates the immune response [2]. Thus, antigen and adjuvant delivery through the lymphatic system can provide an attractive alternative as dendritic cells (DCs) have a significantly larger population in the LN. In a previous report, nanoparticles modified with bile acids were shown to significantly increase oral bioavailability, where they are absorbed in the distal ileum and transported to the intestinal lymphatic system [3]. Nanoparticles modified with bile acids have been applied in the treatment of tumors and diabetes with high oral delivery efficiency by avoiding first-pass metabolism in the liver [3, 4].

Methods

Cationic liposomes were fabricated by thin-film hydration to adsorb protein antigens and double-stranded RNA adjuvants to the surface by electrostatic interaction. Nanoparticles (AAN) were prepared by adding protein antigen and adjuvant solutions to cationic liposomes and coating them with chondroitin sulfate to which glycocholic acid and mannose were separately conjugated. Fluorescently labeled protein antigens and adjuvants were used for microscopic observation of AAN located in the inguinal LNs. Activation of various immune factors (IgG1, IgG2c, IFN-γ and IL-2) and immune cells (dendritic cells, cytotoxic T cells, memory T cells, and regulatory T cells) was evaluated in C57BL/6 mice.

Results

After oral AAN administration, the fluorescently labeled protein antigen and adjuvant were found to colocalize in the inguinal LN for 3-24 hours. AAN showed 1.6-fold enhanced DC maturation over intradermal injection at the same dose size. After weekly oral administration for 4 weeks, plasma concentrations of IgG2c, IFN-γ, and IL-2 were significantly increased compared to normal mice. $CD44^{high}CD62L^{low}$ memory T cell population increased from 2-fold to 2.9- fold over the non-treated group. $CD3^{+}CD8^{+}$ cytotoxic T cells were observed in the two-fold increase in population compared to the non-treated group.

Conclusion

When administered orally, AAN improved DCs maturation efficiency. AAN increased the population of cytotoxic and memory T cells, primarily by promoting Th1 responses. AAN can overcome tumor immunosuppression and inhibit tumor growth and development by reducing regulatory T cells in tumor mouse models. AAN may be applied to prevent tumor recurrence in patients after complete remission and may provide an option for combination therapy with other tumor therapies.

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Effective Pancreatic Cancer targeted photo-immunotherapy using antibody photosensitizer conjugates

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Introduction

In the case of pancreatic cancer overexpressed epidermal growth factor receptor (EGFR), it is known that more than 90 % of KRAS mutations, resulting in a low response rate for antibody therapeutics targeted EGFR. To solve these problems, we adopted photoimmunotherapy system with EGFR antibody conjugated with photosensitizer (EACP) that offers numerous possibilities in eliciting deep tissue penetration ability and boosting immune effect of photosensitizer. EACPs consist of three parts: EGFR specific monoclonal antibody, photoactivatable photosensitizer, and hydrophilic linker. The system with hydrophilic linker prevents photosensitizers from hydrophobic interaction. Therefore, new combined strategy of EACP could achieve a much greater antitumor effect with immune activation and photodynamic effect.

Experimental

For this study, photosensitizers (PSs) and EGFR antibody are conjugated via hydrophilic linker using carbodiimide reaction confirmed by MALDI-TOF/TOF-MS and UV-vis absorbance spectrum analysis. Next, we measured *in vitro* ROS generation efficacy of EACPs using a singlet oxygen sensor green (SOSG) agent compared to free photosensitizers. To confirm *in vitro* cellular uptake of EACPs, flow cytometry and confocal microscopy were performed in pancreatic cancer cell lines with different EGFR expression levels. The cytotoxicity of synthesized EACPs was examined in pancreatic cell lines to confirm their viability at EGFR expression level. To verify body distribution and therapeutic efficacy of synthesized EACPs in tumor-bearing mice, tumor and lymph nodes were collected for immune response analysis.

Results and Discussion

EACPs was characterized by ^{1}H-NMR and UV spectra, absorbance of wavelength appeared at 280 nm which is the peak of antibody. We confirmed the singlet oxygen generation efficacy of EACPs under laser irradiation. This result showed that EACPs overcame the low water solubility of PS, causing well-dispersed state of PSs. *In vitro* cellular uptake behavior of EACPs was confirmed by flow cytometry and confocal microscopy.

Furthermore, EACPs-antigen complex induces DC phagocytosis causing upregulation of DC maturation markers. Additionally, EACPs plus laser accomplished successful tumor regression, enhancing innate immune system linked to adaptive immune response. Thus, the combination of both EACPs and PDT would be provided synergistic effects for pancreatic cancer therapy.

Minimal Invasive Photodynamic Therapy for Obesity-related Type 2 Diabetes

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Introduction

According to foregut theory, foregut have putative signals that are responsible for insulin resistance and abnormal glycemic control. Gastric inhibitory polypeptide (GIP), one of the incretin hormones, has a critical role on adipocyte proliferation and lipid accumulation into adipose tissues, which could be a fetal blow for obese patients. Therefore, the reduction of GIP signal help to alleviate overweight, type 2 diabetes, and to increase glucagon like peptide-1 signal.

GIP level can be controlled by GIP-secreting cell death in duodenum via photodynamic therapy (PDT). PDT is a minimally invasive method that induces oxidative stress and cell death with photosensitizer. Photosensitizers can be accumulated in cytoplasm and generate reactive oxygen species (ROS) under laser irradiation with the specific wavelength. In this study, the potential of PDT in GIP-secreting cells would be confirmed for obesity-related type 2 diabetes.

Experimental

GIP-secreting cells express various types of G-coupled receptors (GPCRs), such as GPR 40, GPR43, GPR 119, and GPR 120, which are activated and stimulated in presence of fatty acid. To recognize GIP-secreting cells, various types of fatty acid were conjugated with hydrophilic polymeric photosensitizer (GIP controllable photosensitizer, GCP). Their target abilities were demonstrated with flow cytometry and confocal fluorescence images in GPR expressed duodenum cells. Under 670nm laser irradiation, intracellular GCP generated ROS in dependence of laser power and effective cell death compared with fatty acid unconjugated photosensitizers.

In C57BL/6 mice induced high fat diet model, GCP was orally administrated, and laser irradiated via endoscopic light device at 670nm wavelength. Their therapeutic effects were confirmed by measuring plasma analysis, body weight loss, and immunohistochemistry.

Results and Discussion

GCP was developed by conjugating C18 fatty acid with hydrophilic photosensitizer through amide coupling mechanism. Duodenal cells more interaction with GCP, compared with non-including fatty acid materials. In modeling mice, orally taken GCP were specially interacted with GIP-secreting cells existed in duodenum and continuously induce cell death via endoscopic light devices. In this result, we demonstrated a decrease of plasma GIP level and losing body weight depending on the specific GIP-secreting cell death.

Suppression of GIP via endoscopic PDT was developed to relieve obesity related T2DM. In further study, they are demonstrated that GIP-secreting cell targeting photosensitizer potentially reduce the main problems associated with conventional endoscopic bariatric surgery via radio-frequency ablation and invasive bariatric surgery. Also, GCP would be a new approach for the future regulation of metabolic syndrome through incretin effect control.

Intranasally-administered Bioresponsive Polyglutamate-based Nanoconjugates for Pediatric Glioma Treatment

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Introduction: Malignant gliomas account for 75% of all brain tumors in children and represent the second leading cause of pediatric cancer deaths after leukemia, with a low survival rate (12-15 months) [1]. This poor prognosis derives from the lack of efficient drug delivery through the blood-brain barrier (BBB) and the associated induces poor efficacy of currently developed therapies. Synthetic polypeptides such as polyglutamate (PGA) represent an efficient therapeutic platform for drug conjugation and the delivery of anticancer drugs or imaging modalities [2], with the benefits of this approach including increased drug solubilization, extended systemic circulation, and the allowance for straightforward surface modification. We selected intranasal administration as a promising non-invasive strategy that enables direct nose-to-brain delivery and bypasses the BBB and hepato-gastrointestinal metabolism. Compared to free drug treatment, polymeric formulations of drugs also enhance permeation through the mucosa. We previously demonstrated that PGA-based polymers represent excellent candidates for brain delivery due to their biodegradability and multivalency, which allows the introduction of drugs and targeting moieties to enhance mucosal permeation [3,4]. Overall, our research focuses on developing novel polypeptide-based therapies using rationally designed stimuli-responsive linkers for targeted drug delivery and release in the brain (Fig. 1).

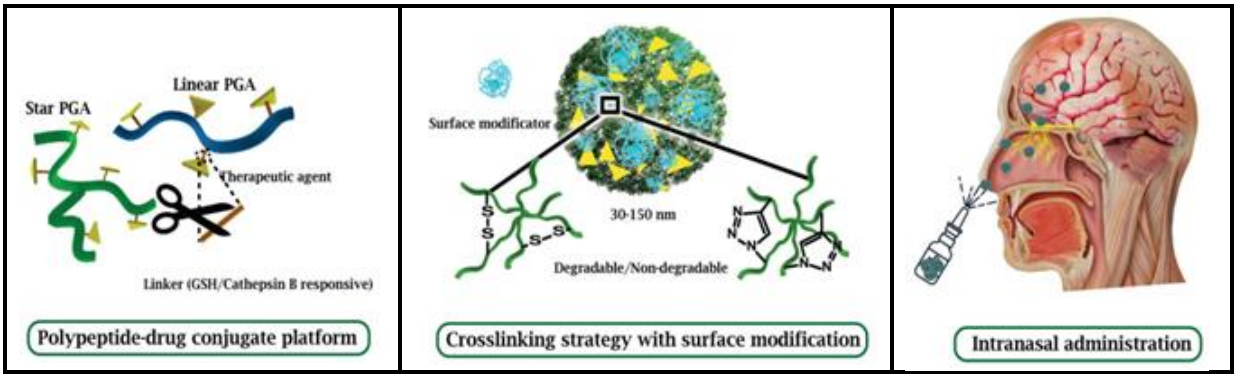

Figure 1. Scheme summarizing our strategy of multivalence polyglutamate-based drug delivery system

Results and Discussion: We designed two stimuli-responsive linkers (redox-responsive disulfide linker and a Cathepsin B-responsive peptidic linker) to conjugate a cyclin-dependent kinase (CDK)4/6 inhibitor to PGA. After adequate purification, we obtained a family of well-defined linear and star-shaped PGA (stPGA) conjugates with a total drug loading of 2 to 15%$_{w/w}$. We analyzed drug release in the presence of dithiothreitol/cathepsin B by RP-HPLC to confirm linker responsiveness. We analyzed conjugate size and conformation by DLS, GPC, CD, and SAXS experiments. Interestingly, we observed delayed drug release for the stPGA conjugates with high drug loading, perhaps due to the compact, organized structure. CD experiments demonstrated

that stPGA conjugates undergo a transition in secondary structure - from random coil to α-helix - upon increased drug loading; however, linear PGAs displayed random coils at all drug loading levels. We performed SAXS experiments to elaborate on this finding, which confirmed the conformational change for conjugates with the highest drug loading. We then evaluated conjugates in patient-derived glioblastoma and diffuse intrinsic pontine glioma (DIPG) cell lines to demonstrate biological activity. The results demonstrated the safety profile of the naked carriers, the concentration-dependent toxicity of conjugates, and the lower cytotoxicity of PGA conjugates compared to free drugs due to altered internalization mechanisms (endocytosis for PGA conjugates and diffusion for free drugs). Notably, observed differences in DIPG cell viability in response to PGA conjugates with different loading confirmed our previous findings that drug loading affects conjugate solution conformation and, therefore, drug bioavailability over time.

We established an intranasal screening platform based on permeation kinetic studies in Franz-cells using sheep mucosa to select the candidates for mucosa permeation. We developed and evaluated different candidates, including (i) linear and stPGA polymers, either naked or modified with a fatty acid, mucopolysaccharides, or lectin peptide; (ii) crosslinked particles (via azide-alkyne cycloaddition or disulfide bonds); and (iii) stPGA incorporated into hydrogel cross-polymer (HA-CP®, developed by PTS, S.L.). We selected two candidates from these test compounds for further in vivo studies, which demonstrated the localization of selected compounds to cells of the olfactory bulb and hippocampus, proving distribution in different brain areas after intranasal administration.

Concluding Remarks: We successfully synthesized a family of star and linear PGA-CDK4/6 inhibitor conjugates with rationally designed disulfide and peptidic linkers that specifically cleave under redox or proteolytic conditions and then undergo self-immolation to release the intact drug. Our studies suggest that drug loading affects conjugate solution conformation and biological output in patient-derived tumor cell lines. In parallel, nanocarriers with efficient nose-to-brain diffusion capacity have been identified; the conjugation of the CDK4/6 inhibitor to these newly identified PGA-based nanocarriers is currently ongoing. Overall, in vitro and ex vivo studies have demonstrated the potential of PGA-based nanocarriers as an effective and efficient treatment for pediatric gliomas.

Acknowledgments: The authors acknowledge E. Solano and M. Malfois from BL-11 NCD-SWEET beamline at ALBA Synchrotron for their support with SAXS experiments. We thank the Asociación Española contra el Cáncer (TM PhD grant PRDVA172014MELN, ICS **Junior** AECC grant INVES211323CONE), Spanish Ministry of Economy (PID2019-108806RB-I00), Generalitat Valenciana, European Regional Development Fund (PO FEDER Comunitat Valenciana 2014-2020), and La Xocolatà funding for financial support.

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Nano-Complexation of Glatiramer and Diclofenac integrated into In-situ Nasal Gel Synergistically Enhances Re-myelination in a Mouse Model of Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination and inflammation of the central nervous system. Delaying the progression of the disease is still an unmet need. Glatiramer acetate (GA) is an immunomodulator approved for the management of MS through peripheral mechanisms. Diclofenac sodium (DCL) is a common non-steroidal anti-inflammatory drug. Direct nose-to-brain drug delivery is a valuable strategy for escaping the blood-brain barrier and delivering therapeutics to the brain. The study aimed to examine the neuroprotective effect of intra-nasally administrated GA-DCL nano-complexation against cuprizone (CPZ)-induced demyelination in mice.

GA-DCL nanoconjugate was formulated by ion-pairing at different molar ratios using Box-Behnken design. The optimized formula was characterized and incorporated into gellan gum in-situ gel. The treatments were administrated intra-nasally while toxic CPZ was given by oral gavage once daily for six weeks. Animals (n=60) were divided randomly into five groups (control, CPZ, CPZ+DCL, CPZ+GA, CPZ+GA-DCL). All mice were subjected to behavioural tests. Brain corpus callosum and cortex tissues were investigated by histopathological, biochemical, and molecular evaluations.

The optimized GA-DCL was prepared at (1:2.7) molar ratio, which exhibited particle size of 198.21 nm and zeta potential of 34.53 mV. Animal studies revealed that optimized GA-DCL showed superior protective activities than either drug alone. It significantly reversed CPZ-induced body weight loss and rescued motor dysfunction. Histologically, it showed neuroprotection evidenced by great remyelination. It also inhibited CPZ-induced oxidative stress. It was associated with inhibiting the inflammatory markers IL-6, TNF-α, COX-2, and NF-κB secretion. Western blot analysis confirmed the superior remyelination properties of GA-DCL as it significantly enhanced proteins concentration of PDGFR-α, BDNF, Olig2, and MBP.

Overall, nano-complexation of GA to DCL in an optimized formula exhibited excellent physicochemical properties and significantly enhanced the neuroprotective properties of GA. This is attributed to, at least partly, enhanced antioxidant, anti-inflammatory, and remyelination activities.

Hydrogel-Based Delivery of Nonviral-Engineered Mesenchymal Stem Cells for Treating Spinal Cord Injury

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Spinal cord injury (SCI) is a serious and irreversible trauma. Its causes include traffic accidents, falls, violence, sports activities, and cancer. Nearly half of SCI patients fall into the situation of lifelong permanent quadriplegia, which seriously affects their quality of life and also brings a heavy medical burden. However, current treatment methods include spinal decompression surgery, spasticity treatment, and rehabilitation treatment, which cannot effectively restore neurological deficits. In recent years, stem cell therapy has shown great potential on SCI by promoting neuron repair and regeneration. Despite its great potential in SCI treatment, stem cell therapy is still in its infancy stage and requires improvements in various aspects. For example, after stem cell implantation, low cell retention and cell activity are often caused by the harsh wound microenvironment leading to less effective therapeutic outcomes. To address this challenge, we propose a new injectable self-healing hydrogel formulation (shHA-GEL-GC) for stem cells transplantation and improve the survival and retention time in the injured area. Besides developing hydrogel to improve cell delivery efficiency, a nonviral magnetic gene delivery technique was utilized to construct human mesenchymal stem cells overexpressing vascular endothelial growth factor (VEGF) (^{VEGF}hMSCs) to promote the SCI repair process. Mechanical strength, porosity, and degradability of the shHA-GEL-GC can be controlled by varying the polymer compositions. Good cell viability of the encapsulated stem cells was confirmed after needle injection. Accumulative release of VEGF from the ^{VEGF}hMSCs-laden shHA-GEL-GC hydrogel was confirmed and the secreted VEGF effectively promoted the proliferation of vascular endothelial cells. After injecting ^{VEGF}hMSCs-laden shHA-GEL-GC into the animal SCI area, good cell retention and survival were confirmed by the in vivo imaging system (IVIS). In the SCI animal model, we demonstrated that the ^{VEGF}hMSCs-laden shHA-GEL-GC hydrogel not only enhanced the angiogenesis in the injury area but also improved the recovery of animals six weeks after the surgery. Our findings suggested ^{VEGF}hMSCs-laden shHA-GEL-GC hydrogel holds great promise on SCI treatment.

Hierarchically targetable polysaccharide-coated solid lipid nanoparticles as an oral chemo/thermotherapy delivery system for local treatment of colon cancer

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Introduction

Although oral formulations of anticancer chemotherapies are clinically available, the therapeutic action relies mostly on drug absorption, being inevitably accompanied with systemic side effects. It is thus desirable to develop oral therapy systems for the local treatment of colon cancers featured with highly selective delivery to cancer cells and minimized systemic drug absorption. The present study demonstrates the effective accumulation and cell uptake of the doxorubicin and superparamagnetic iron oxide nanoparticles-loaded solid lipid nanoparticles (SLNs) delivery system for chemo/magnetothermal combination therapy at tumors by hierarchical targeting of folate (FA) and dextran coated on SLN surfaces in a sequential layer-by-layer manner. Both the in vitro and in vivo characterizations strongly confirmed that the dextran shells on SLN surfaces not only retarded the cellular transport of the FA-coated SLNs by the proton-coupled FA transporter on brush border membranes in small intestine, but also enhanced the particle residence in colon by specific association with dextranase. The evaluation of the *in vivo* antitumor efficacy of the hierarchically targetable SLN therapy system by oral administration showed the effective inhibition of colon tumors.[1]

Experimental

The SLNs were prepared by double emulsion method and sequentially coated with folate-modified D-α-Tocopherol polyethylene glycol 1000 succinate (FA-TPGS) and octadecanol-conjugated dextran (Oct-Dex) by hydrophobic interaction. The enzymatic degradability of dextran was demonstrated by the incubation with dextranase, a bacteria-produced glucanohydrolase presents exclusively in colon. The cellular uptake examinations were performed by FA receptor (FAR) overexpressing CT26 colon cancer cells. An orthotropic CT26 colon tumor model was established with BALB/c mice. For *in vivo* distribution in gastrointestinal tract (GI tract), SLNs were labeled with NIR probe, DiI, to facilitate IVIS fluorescence detection (Xenogen IVIS system). For *in vivo* tumor inhibition study, mice were treated with drugs by gavage at a daily DOX dosage of 12 mg/kg for total three doses. To generate hyperthermia effect, the high-frequency magnetic field (HFMF) treatment was performed for 7 min at 6 h post gavage.

Results and Discussion

The dextran/FA-modified SLNs (DFSLNs) exhibited the average particle sizes of 132.1 nm and the DOX loading content of 9.27 wt%. The incubation of DFSLNs with dextranase led to the reduction of nanoparticle sizes to 97 nm, similar to the sizes of FA-modified SLNs (FSLNs). As an oral formulation, the colloidal stability and drug leakage of this SLNs were examined in simulated gastrointestinal fluids. The results demonstrate that the particle sizes of the SLNs remained essentially unchanged over 8 h and slow DOX release profiles, 22% for FSLNs and DFSLNs and 10% for SLNs and DSLNs, were attained over 24 h. The *in vitro* targeting efficiency of the FA-decorated SLNs to FAR overexpressing CT26 cells substantially enhanced compared to TSLNs (control group) and DFSLNs (**Figure 1**). The reduction of cellular uptake of DFSLNs was caused by the shielding effect with dextran that impaired the FA-mediated interactions between

the NPs and the cells. As a consequence, after the removal of dextran coating with the enzymatic action of dextranase, the DFSLNs showed comparable uptake to FSLNs.

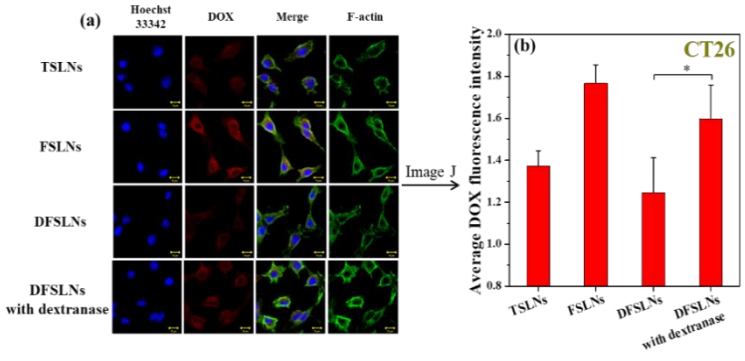

Figure 1. (a) Confocal laser scanning microscopy images of CT26 cells after treatment with SLNs. Nucleus and cytoskeleton were stained with Hoechst 33342 and F-actin, respectively. Scale bars: 10 mm. (b) Average DOX fluorescence intensity analyzed by Image J. *P < 0.05. Error bars represent mean ± s.d.

The *in vivo* distribution of DFSLNs in GI tract shows reduced retention in intestine than that of FSLNs due to the FA residues sequestered by dextran coating (**Figure 2**). Moreover, DFSLNs promoted colon tumor region accumulation for about 2.8-folds compared to FSLNs in part by evading the undesired biorecognition. In particular, the effective tumor growth inhibition of the orthotopic colon tumor for the tumor-bearing mice receiving the DFSLNs and DFSLNs/HFMF treatments, respectively, clearly demonstrated the prominent therapeutic efficacy of DFSLNs for local treatment by oral administration.

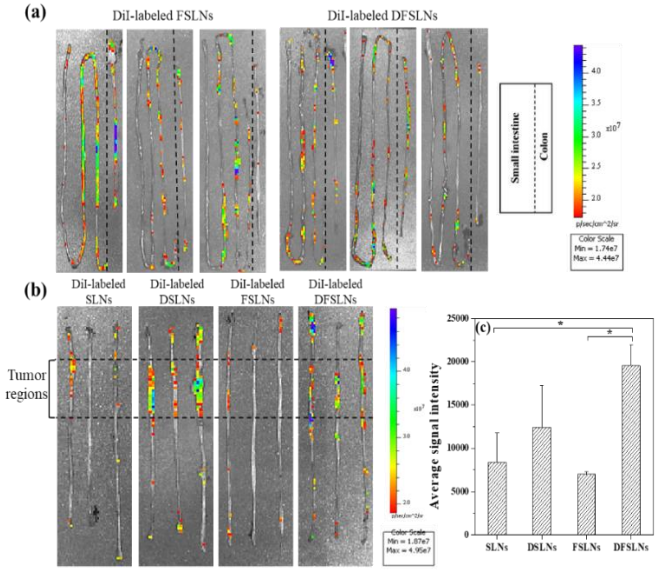

Figure 2. IVIS images of (a) small intestine and colon collected from orthotopic CT26 tumor-bearing mice at 6 h after the gavage of SLNs. (b) Accumulation of DiI-labeled SLNs within the tumor region via IVIS imaging. The average signal intensity was obtained from the fluorescence signal intensity of ROIs in the images. *P < 0.05. Error bars represent mean ± s.d (n=3).

Conclusion

The selective, enhanced accumulation of DFSLNs at colon tumor sites confirmed the successful development of a hierarchical targeting formulations for the oral delivery of dual modality local therapy against colon cancer. DFSLNs cannot only evade the systemic absorption via avoiding biorecognition in small intestine, but also enhance the accumulation in colon by colon enzyme-responsive dextran. Because of the restricted therapeutic action of DFSLNs therapy delivery system on the local tumor sites, no apparent systemic side effects were observed.

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Optimized Delivery of MCL-1 siRNA in a Breast Cancer Cell Model In Vitro

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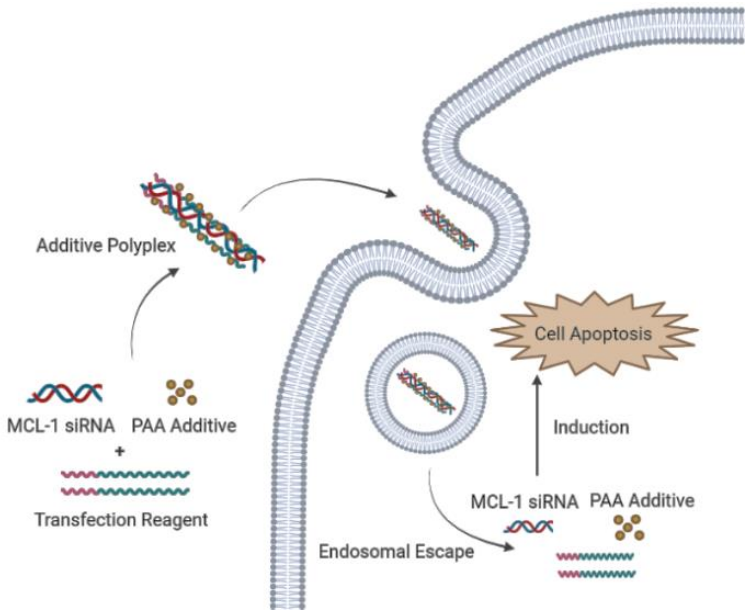
Abstract

This research aims to optimize a system for siRNA-mediated silencing in breast cancer cell types. A lipopolymeric transfection reagent was used in this study with a suitable additive in the formulation. Several conditions such as the siRNA to polymer weight ratio, siRNA concentration, cell density, and the type of transfection reagent were found to affect the cell growth inhibition in breast cancer cells. In addition, MCL-1 siRNA was found to limit the proliferation of breast cancer cells and, under optimal conditions, MCL-1 siRNA delivery can achieve low cell viability (30%) compared to the non-treatment group, and show statistically significant difference compared to the scrambled siRNA-treated group. Furthermore, when MCL-1 and survivin siRNAs were combined, they had a more promising inhibitory effect on MDA-MB-436 cell proliferation than a single siRNA, where the cell viability was shown at ~20% for the combination of siRNAs. This research has de potential to be developed to target specific anti-apoptotic genes for various treatments. Furthermore, the transfection reagents could be modified to harbor other siRNAs in order to create novel treatments for cancer and other illnesses.

Graphical Abstract



Oral Nanoparticles to Treat Obesity

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Introduction: More than 70% of American adults are overweight or obese, a precondition leading to chronic diseases, including diabetes, arthritis, and hypertension. Among other factors, diets with high fat and cholesterol content have been implicated in obesity. We hypothesize that trapping the fat and cholesterol molecules, that has been taken with food, within the gut and preventing them from being absorbed by intestine, can a potential treatment modality for obesity. Therefore, we have screened a series of molecules, and investigated their feasibility of trapping fat molecules within it, in presence of water. We have observed that a cationic charged ionic liquid (IL) has potential to interact with fat molecule and form micrometer size particles in presence of water. We have also observed that the same IL has potential to enhance intestinal transportation of hydrophilic molecules such as insulin, GLP1 even leptin monoclonal antibody.

Experiments: To investigate in vitro feasibility, we have co-incubated the materials, an omega-3 fat molecule in presence of water, that mimic gastrointestinal track. To investigate intestinal diffusion, we have taken these mixtures within the lumen of intestine and observed diffusion. For in vivo studies, we have taken a group a SD rat and fed with high fat diet (HFD) (contain 20% more fat than regular food). The rats were dosed with various IL containing particles and their body weight were compared with another group of rats that have not been treated but fed HFD. Further, to expedite the body weight reduction for individual mediated with leptin resistant, we have prepared an anti leptin antibody (mAb) particle and delivered orally to the obese rat.

Results: We have observed that the mixture if IL, fat molecule and water forms a large size particle within a moment, a clear indication that the IL has potential to trap the fat molecules and prevent them from being transported through intestinal membrane. In the in vivo studies, we have observed that the rat treated with IL gain significantly less body weight compared to untreated group. More interestingly, the rat that have been treated with metal particle (MP) with IL and IL+leptin reduce their body weight by 20% and 30% within 4 weeks, compared to that of rat treated with free IL or untreated.

Conclusion: We conclude that oral dose of IL can slow down the body weight gain, but IL mediated particles such as MP+IL and IL+leptin mAb delivery can even result reduction of body weight. With further development and investigation, such oral nano-formulation could be considered as an effective medication for treating diet induced obesity.

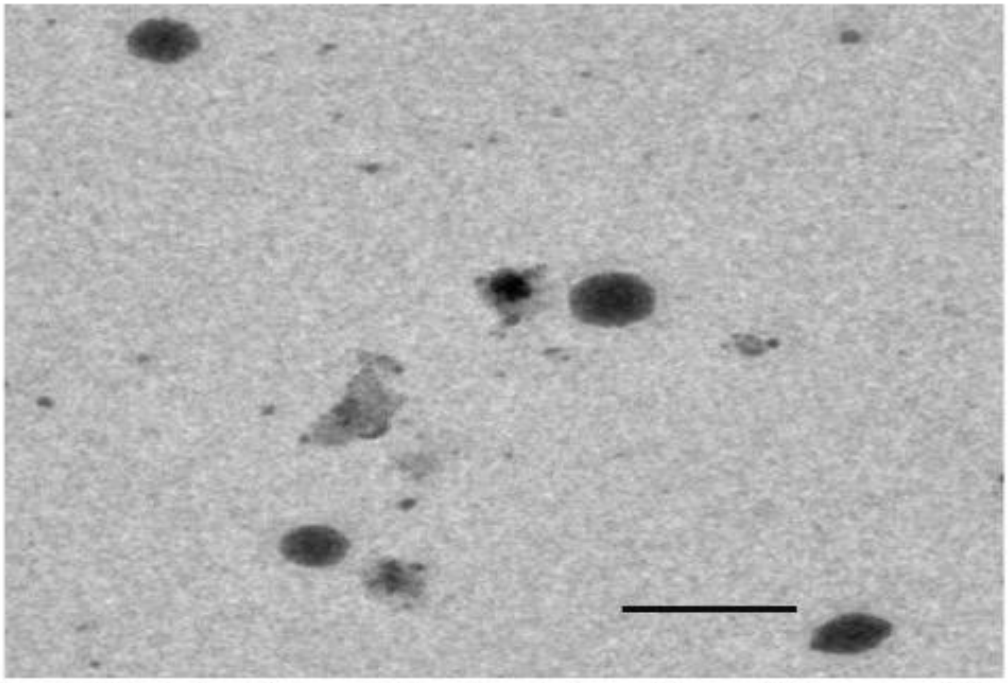

Figure 1. Coincubation of IL, Omega-3 fatty acid and water result large particle formation which is 4-5 μm in diameter. Scale bar represent 5 μm.

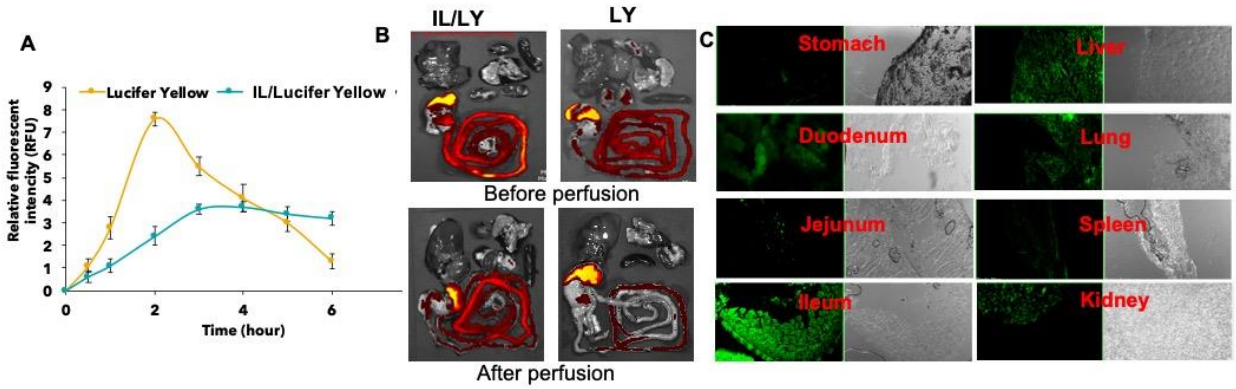

Fig. 2. Biodistribution of orally administered fluorescent dye via capsule and GFP expression. *(A) To be able to quantify the absorption and biodistribution, lucifer yellow labeled plasmid containing tablet was orally admonished and presence of lucifer yellow was measuring using UV spectroscopy, (B) Fluorescent molecular tomography images shows presence of fluorescence dye 6h or post administration. High intensity in small intestine even after perfusion indicate strong adhesion with the intestinal membrane. (C) expression of GFP in various organ besides small intestinal membrane also indicate the the ability of IL to enhance oral absorption of large molecules biologics.*

Efficient brain delivery of multifunctional polymer-conjugated lentivirus for epilepsy therapy

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Abstract: Gene therapy approaches have been initiated as new era of treatment for epilepsy. However, the administration route and efficient delivery of the transgene-vehicles to brain have been challenging. To overcome these obstacles, lentivirus-expressing wild type deoxyhypusine synthase ($DHPS^{WT}$) was chemically modified to compensate for the mutated DHPS forms found in neurodevelopmental disorders with epilepsy. Further, to generate lentivirus-PPA complex enabling efficient nose-to-brain delivery, it chemically conjugated with pH- and reduction-responsive copolymer [poly(ethylene glycol)-poly(bioreducible β-aminoester)-arginine-grafted polyethyleneimine (PPA)]. The optimized lentivirus-PPA complex was effectively delivered to the brain tissues via intranasal administration, suggesting its ability to bypass the Blood-Brain Barrier (BBB) and allow efficient $DHPS^{WT}$ expression. Therefore, intranasally deliverable lentivirus-PPA complex can offer a promising gene therapy tool that can correct neurodegenerative malfunction associated with epilepsy.

Systemically administered adenovirus coated with active tumor targeting polymer

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Abstract: Cancer gene therapy using oncolytic adenovirus (Ad) has been highlighted as a promising cancer therapeutics. However, systemic delivery of oncolytic Ad has limitations concerning the immunogenicity and hepatic tropism of Ad. To overcome these limitations, we have synthesized folic acid (FA)-conjugated PNLG variants (P_5N_2LG-FA and P_5N_5LG-FA) with 5 kDa poly(ethylene glycol) (PEG) and varying level of cationic charge as tumor-targeted systemic delivery tool for adenovirus (Ad). Our findings demonstrate that green fluorescent protein (GFP)-expressing Ad complexed with P_5N_2LG-FA or P_5N_5LG-FA (Ad/P_5N_2LG-FA or Ad/P_5N_5LG-FA, respectively) induced higher transduction efficiency compared to naked Ad or Ad complexed with control polymer lacking tumor targeting moiety (Ad/P_5N_5LG) in folate receptor (FR)-overexpressing cancer cells (KB and MCF7). FA-conjugated nanocomplexes (Ad/P_5N_2LG-FA and Ad/P_5N_5LG-FA) demonstrated enhanced cell uptake via specific interaction of FA-FR. Importantly, systemically administered Ad/P_5N_5LG, Ad/P_5N_2LG-FA, and Ad/P_5N_5LG-FA exhibited significantly higher retainment of the virus in blood circulation, lower level of hepatic sequestration and higher level of intratumoral accumulation. Collectively, these findings demonstrate that careful optimization of polyplex surface charge is critical to successful tumor-targeted systemic delivery of Ad nanocomplexes.

Polyethylenimine derived lipopolymers efficiently transfect siRNA in human peripheral blood mononuclear cells

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Human peripheral blood mononuclear cells (PBMCs) are vital components of the innate immune system. Signal Transduction and Activator of Transcription 5A (STAT5A) is one of the major transcription factors in these cells, which affects the regulation of multiple downstream genes and cell fate. Manipulating STAT5A expression in PBMCs is a crucial means to develop therapies for several diseases, including cancers and inflammatory diseases. Small interfering RNA (siRNA) is used to downregulate any gene and reduce the target protein level. Therefore, silencing STAT5A by siRNA delivery can be a key player to therapeutic development. However, the development of clinically effective siRNA carriers is needed since siRNA molecules are highly unstable in physiological fluids and their anionic nature prevents them from traversing cellular membranes. Another challenge is individual cells vary among different individuals due to differences in genetic profiles[1]. Thus, the success of siRNA therapy will be governed by the efficiency of the delivery system. In this study, we explored lipid substituted polyethylenimines (PEI) to deliver siRNA molecules to PBMCs from healthy volunteers. Our current study is aimed at assessing the siRNA delivery efficiency of these non-viral polymeric carriers.

The preparation of polymeric nanoparticles was described previously [23]. We prepared 3 polymers for this study by substituting low molecular weight PEI with linoleic acid (PEI-LA), cholesterol (PEI-Chol), and lauric acid (PEI-Lau). Fresh peripheral blood samples were collected from twelve healthy adult human volunteers and supplied by Canadian Blood Services (ethics approved secured by the U. of Alberta Ethics Board). PBMCs were isolated with the help of Ficoll-Paque™ PLUS media. For transfection, STAT5A siRNA (siSTAT5A) were complexed with the lipopolymers while complexes of scrambled siRNA (CsiRNA) with lipopolymers were used as the negative control group. The polymers were added to siRNAs at weight ratios of 7.5:1 for PEI-LA and PEI-Lau, and 12:1 for PEI-Chol. After incubation for 30 min at room temperature, complexes were added with PBMCs in triplicate. After 3 days of siRNA transfection, cells were collected for RNA isolation with TRIzol™. Real-time quantitative PCR (RT-qPCR) gene expression analysis was performed to assess the expression level of STAT5A in individuals. Changes in STAT5A expression were normalized to reference gene, □-actin and comparing with non-treated samples.

We observed different STAT5A downregulation levels in healthy donors. Among the three nanoparticles studied, PEI-Chol and PEI-Lau substituted polymers showed better siRNA delivery. PEI-Lau gave 8 significant transfections and PEI-Chol exhibited 6 significant transfections among 12 donor PBMCs. PEI-LA provided only 3 significant silencing among the donor cells. We did not get any significant results from 3 donor cells. Based on the analysis of average silencing efficiency, PEI-Chol helped to downregulate STAT5A expression by 56.8% ($P < 0.0005$), and PEI-Lau by 40.2% ($P < 0.005$). Our future work to explore the use of these polymeric carriers in treatment of experimental leukemias is currently underway.

In conclusion, lipid substituted PEIs efficiently transfected siRNAs and helped to knock down the STAT5A level significantly in PBMCs from healthy donors. These polymers can be used as an effective non-viral carrier to deliver RNA therapeutics into human primary cells. More studies are required to evaluate the immune response levels and changes in downstream targets.

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Polymeric Micelles: Thinking beyond solubility and towards drug retention

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The focus of polymeric micelle technology to date has been on increasing the solubility of poorly soluble drugs to improve drug delivery, widen the therapeutic window, and improve efficacy. Here we outline our efforts to improve efficacy and the delivery of drugs to tumors by focusing on drug retention in the micelles, rather than just solubility. Improved drug retention in the polymeric micelle leads to improved tumor drug exposure and therapeutic outcomes in triple negative breast cancer. We have designed a novel assay to measure drug partitioning *in vitro* which can be correlated to drug disposition *in vivo*. Our studies indicate that drug partitioning between protein and micelles is dependent on polymer/drug ratio, drug concentration, and the choice of polymer. Taken together, this *in vitro* assay could be used for the *in vitro* optimization of formulations, and when combined with pharmacokinetic modeling, can reduce the time and money required to complete *in vivo* preclinical evaluations. These efforts will lead to improved preclinical work flow and improve the efficiency of formulation translation to the clinic.

Polymeric nanoparticle delivery of CDK4/6 inhibitor for treatment of medulloblastoma and a combination with mTOR inhibitor elucidated by scRNA-seq

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Patients with medulloblastoma, the most common malignant brain tumor in children, need new therapies for both upfront treatment and treatment after recurrence. The CDK4/6 selective inhibitor palbociclib, which is known to arrest cells in the G1-phase of the cell cycle and blocks RB phosphorylation, has shown anti-tumor activity in patient-derived xenograft (PDX) mouse models of medulloblastoma. However, palbociclib therapy for medulloblastoma have consistently been limited by tumor recurrence. Here, to improve palbociclib efficacy for brain tumor therapy, we developed novel poly(2-oxazoline) (POx) based amphiphilic block copolymer for encapsulation of palbociclib in polymeric nanoparticle (POx-palbo). Various structures of poly(2-oxazoline) triblock copolymer were synthesized and palbociclib solubilization in the copolymers was screened. Triblock copolymer composed of poly(2-methyl-2-oxazoline), poly(2-n-butyl-2-oxazoline), and poly(2-caboxyethyl-2-oxazoline) showed palbociclib solubilization with high loading up to ~28 % and formation of nanoparticle with size of 55 nm (PDI < 0.1). The drug delivery to brain was also dependent on drug loading and polymer structure. We tested POx-palbo in a genetically-engineered mouse model of SHH-subgroup medulloblastoma. POx-palbo reduced toxicity and improved pharmacokinetics, pharmacodynamics and efficacy of palbociclib while free drug failed to improve survival outcomes. We further investigated the effects of chronic CDK4/6 inhibition using immunofluorescence and single-cell RNA-seq to identify potential co-targets and then tested combinations of POx-palbo with additional agents. Tuning polymer structure for palbociclib delivery enabled high dose therapy *in vivo*, enhanced drug exposure to target site resulting in the dramatically improved survival outcomes.

PEGylated Functional Upstream Domain Peptide Targets Fibronectin Assembly and Possesses Enhanced Tumor Exposure in a Murine Breast Tumor Model

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Breast cancer is a type of solid tumor in which dynamic crosstalk between cancer cells, stromal cells, and the extracellular matrix (ECM) play an important role in disease progression. Deposition of ECM is mediated by activated fibroblasts and requires fibrillization of fibronectin, which precedes and controls the formation of other ECM proteins. Therefore, abnormal accumulation of fibronectin can induce aberrant deposition of ECM in breast cancer, leading to tumor fibrosis and unsatisfactory treatment outcomes. The Functional Upstream Domain (FUD) peptide is a potent fibronectin assembly inhibitor that binds to the N-terminal 70-kDa domain of fibronectin with nanomolar binding affinity. However, the FUD peptide shows rapid renal clearance and limited *in vivo* accumulation mainly due to its 6-kDa size. We have PEGylated the FUD peptide (PEG-FUD) to enhance its bioavailability and pharmacokinetic properties. In this study, we investigated *in vitro* and *in vivo* biodistribution of the peptides, especially focusing on breast cancer. *In vitro* fluorescence imaging shows that both FUD and PEG-FUD can bind to the fibronectin matrix assembled by cancer-associated fibroblasts. In addition, *in vivo* imaging techniques confirmed that fluorescently-labeled PEG-FUD preferentially target tumor with prolonged tumor accumulation in a mouse model of breast cancer. These results suggest that PEG-FUD has the potential as an anti-fibrotic therapeutic as well as a targeting agent with an improved pharmacokinetic profile.

Pharmacokinetics and biliary excretion of a paclitaxel prodrug-loaded polymeric micelle drug delivery system

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Abstract: The taxanes remain an important class of chemotherapeutics used for the treatment of a variety of solid tumors. Paclitaxel (PTX) in particular is a potent microtubule stabilizer frequently prescribed to breast, lung and ovarian cancer patients. However, the hydrophobicity of paclitaxel presents challenges for formulating the drug for intravenous injection, and toxic co-solvents are required for drug solubilization. Nano-sized polymeric micelles have been proposed as safer drug delivery systems for hydrophobic drug entities. In order to improve the carrier stability following injection and reduce off-target toxicities, we have developed a novel paclitaxel prodrug conjugated with 8 lactic acid units (o$(LA)_8$-PTX) for enhanced compatibility with the hydrophobic core of poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA) micelles. Improved drug-carrier compatibility translated to greater antitumor efficacy and reduced toxicity in multiple murine cancer models versus unmodified PTX-loaded micelles.

This poster will focus on a recent investigation of the pharmacokinetic profile of o$(LA)_8$-PTX-loaded micelles in bile duct cannulated Sprague Dawley rats performed in collaboration with the NIH's Nanotechnology Characterization Lab (NCL). Plasma and bile analysis by liquid chromatography mass spectrometry (LCMS) revealed o$(LA)_2$-PTX and o$(LA)_1$-PTX to be the dominant metabolite species with low concentrations of parent drug PTX. Previous work in our group has proved these two metabolites to be bioactive, indicating full conversion to PTX is not a requirement for drug action. As a result, plasma area under the curve (AUC) of PTX generated from o$(LA)_8$-PTX was significantly lower than that generated from the marketed nano-formulation, Abraxane.

Liposome Drug Market Overview and Insights to Liposomal Drug Development in The Aspect of Regulatory Guidance

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College of Pharmacy, California Health Science University, Clovis, CA, USA

The global liposomal drug market has been increased dramatically with a market growth rate of 13.2%, and its market size is predicted to be worth about $6993 million by 2027. As an intrinsically complex delivery system, liposomal drugs face much greater characterization and regulatory review challenges than traditional small molecule drugs and even biologics. Due to rapid liposomal drug development, both European Medicines Agency (EMA) and US Food and Drug Administration (FDA) have developed and published regulatory guidance of required information for new liposomal drug applicants to submit for licensing. This poster will discuss the current global liposome drug market, the driving force of increased research and development (R&D) in liposomal drug delivery systems, significant factors would further boost the liposomal drug market growth, review and compare EU and US regulations on liposomal drugs, and provide insights to strategies through entire liposomal drug development in terms of regulatory affairs. The presented content fills the gap between diligent R&D research work in the laboratory and the rapid increase of liposomal drug market growth and its regulatory guidance development.

Local application of biodegradable dexamethasone-loaded hydrogel improves motor cognitive functional recovery of after traumatic brain injury in rats

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Background: Sustained neuroinflammation causes progressive damage to the neural network and severely limits functional recovery following traumatic brain injury (TBI) [1]. Steroidal anti-inflammatory drugs, such as the dexamethasone (DX) can minimize neurotoxic inflammation by controlling the early expression of cytokines/chemokines responsible for microglia and macrophage activation [2]. In our previous work, we have developed a hydrolytically degradable hydrogel composed of polyethyleneglycol-bis-(acryloyloxy acetate) (PEG-bis-AA) and dexamethasone (DX)-conjugated hyaluronic acid (HA-DXM) and demonstrated that local application of PEG-bis-AA/HA-DXM hydrogels reduces neuroinflammation, apoptosis, and lesion volume and improves neuronal cell survival and motor functional recovery at 7 days post-injury (DPI) in a rat controlled cortical impact (CCI) TBI model *in vivo* [3]. In this study, we evaluated the effect of PEG-bis-AA/HA-DXM hydrogels on motor and cognitive function and aspects of secondary injury, such as lesion volume, inflammatory response, apoptosis, and neuronal survival at 14 DPI in a rat CCI TBI model.

Methods: PEG-bis-AA/HA-DXM hydrogels were synthesized as previously described [3]. To generate a mild CCI TBI model in rats we first performed a 5 mm craniotomy and impacted the cortex with a TBI impactor (Precision Systems and Instrumentation) using a flat 3 mm diameter impacting tip at a set speed of 3.5 m/s, depth of 2 mm, and dwell time of 250 msec. Animals were divided into 3 groups: 1) Sham group (uninjured, craniotomy only), 2) TBI (untreated) group, and 3) TBI treated with PEG-bis-AA/HA-DXM hydrogel (n=8 animals /group). Motor function was assessed by accelerating rotarod (4 to 40 rpm in 90 sec) at 1, 3, and 6 days post-injury. Cognitive function was evaluated by Morris Water Maze (MWM) tests for spatial learning. A circular water maze tank (183 cm diameter) was filled with water (maintained at 25°C) and a circular escape platform (15 cm diameter) were submerged 2 cm below the water surface. Starting at 8 DPI, the animals were trained to find the platform within 60 seconds for 5 consecutive days (4 training trials/day) and swim paths were recorded using a video tracking system and latency time (sec) to reach the platform measured. At 14 DPI after cognitive function study, animals (n=3 rats/group) were sacrificed by decapitation and total RNA were isolated from fresh brain tissue and cytokine mRNA expression levels measured by RT-PCR. For histological analysis, we sacrificed rats (n=5 rats/group) at 14 DPI via cardiac perfusion using saline followed by 4% PFA under deep anesthesia. Brains were retrieved, fixed, frozen, and cryo-sectioned at 30 μm thickness. Coronal brain sections were stained with Nissl for cavity size measurements and by IHC for ED1 and

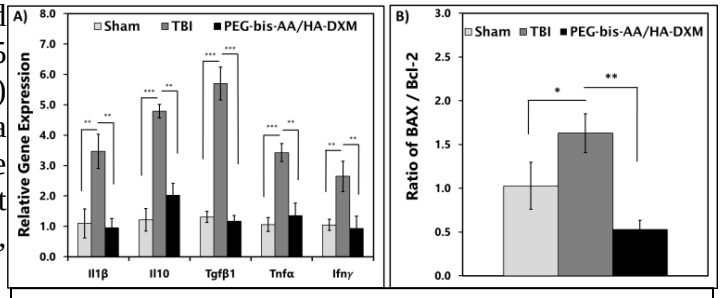

Figure 1. Effect of PEG-bis-AA/HA-DXM hydrogels on inflammatory cytokinelevel and apoptotic gene expression in mRNA level. Relative mRNA expression levels of cytokines (A): IL1b, TGFb1, TNFa, IL10, and INFγ and the ratio of BAX/Bcl-2 (B). Data represent the mean ± SD (n=3/group). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Arg1 for M1 and M2 macrophages and NeuN for neuronal nuclei.

Results: In RT-PCR analysis, we observed that PEG-bis-AA/HA-DXM hydrogel treatment significantly reduced levels of the inflammatory cytokines IL-1β, TGF-β1 TNF-α, IL-10, and IFNγ and increased the ratio of BAX/Bcl-2 expression compared to the untreated TBI group at 14 DPI (Fig. 1). By histological analysis, we observed that hydrogel treatment significantly reduced inflammatory cell (ED1+ cells) and apoptosis (TUNEL+ cells) compared to untreated animals at 14 DPI (Fig. 2). We also observed that hydrogel treatment significantly reduced the lesion volume and increased neuronal survival (NeuN+ cells) compared to untreated TBI group at 14 DPI. For the motor function study, we observed that PEG-bis-AA/HA-DXM treated animals exhibited improved motor function compared to untreated TBI at all time points and a significant difference were observed at 6 DPI by the rotarod test (Figure 3A). For the cognitive function study, latency to find the platform was faster in the hydrogel-treated group compared to the untreated TBI group at all time points and significant difference was observed at 12 DPI time point (Figure 2B).

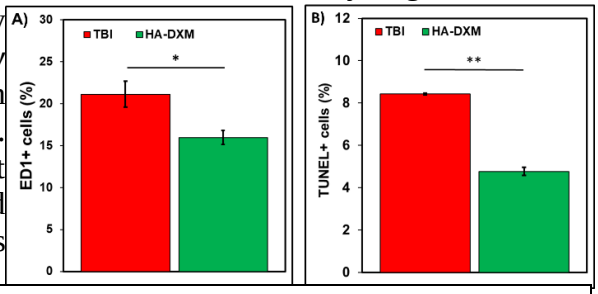

Figure 2. Effect of PEG-bis-AA/HA-DXM hydrogels on inflammatory response and apoptosis by histological analysis.The average % CD68 (ED1)+ cells (A) and %TUNEL + cells.
(n=5 rats/group, n=3 sections/rat
***p* < 0.05 compared to untreated TBI group.**

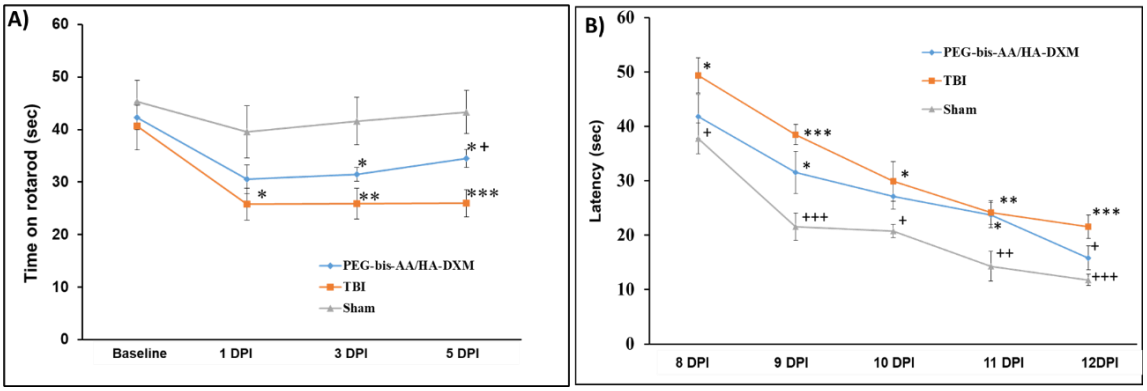

Figure 3: (A) Motor function was assessed by rotarod test at 1, 3, and 6DPI. Hydrogel treatment led to significant improvement in rotarod performance. (B) Cognitive function was evaluated by a MWM test for spatial learning at 8 DPI and continuing for 5 consecutive days. (n=8 rats /group). * p<0.05 compared to Sham, ** p<0.01 compared to sham, *** p<0.001 compared to sham. + p<0.05 compared to TBI, ++ p<0.01 compared to TBI, +++ p<0.001 compared to TBI.

Conclusion: PEG-bis-AA/HA-DXM hydrogel treatment immediately after injury can reduce inflammatory response and the anti-inflammatory effect of hydrogel attenuated brain damage and improved neuronal cells survival after TBI. The results of this study indicate significant benefits in motor and cognitive functional recovery from local application of PEG-bis-AA/HA-DXM hydrogels up to 2 weeks post-injury.

Acknowledgement: This work was supported by the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) [grant number 5P20GM103444-07].

References: [1] Kumar, A. and Loane, D.J. *Brain Behav. Immun.* (2012); [2] Zhang, Z., et al. *Acta Neuropathol.* (2007) ; [3] Jeong, D. et al. *Biomedical Materials* 16, 1-14 (2021)

Rolipram delivered by PgP nanocarrier enhances motor function and reduces neuropathic pain in a rat contusion SCI model

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Statement of Purpose: Traumatic spinal cord injury (SCI) is a major source of morbidity and mortality worldwide. SCI disrupts axonal pathways, leading to permanent motor, sensory, and autonomic dysfunction, as well as chronic pain, respiratory impairment, and loss of bowel or bladder control. Several complex pathophysiological mechanisms such as the reduction of cyclic adenosine monophosphate (cAMP), activation of myelin-associated inhibitors and formation of astrogliosis limit spontaneous recovery following SCI. Our long-term goal is to develop a combinatorial therapy, using a cationic amphiphilic polymeric nanocarrier, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP, US patent 10,232,050B1) for co-delivery of rolipram (Rm) and RhoA siRNA (siRhoA) modulating the cAMP andRhoA signaling pathways, respectively, with L1 neural cell adhesion molecule as a targeting moiety. In previous studies, we demonstrated that a single intraspinal injection of Rm-PgP nanoparticles restored the cAMP level and increased neuronal cell survival and reduced inflammatory response in a rat severe compression SCI model [1]. In this study, we evaluated the effect of Rm-PgP single and repeat injection via intrathecal catheter on neuroprotection and inflammatory response; motor function recovery; and neuropathic pain in a rat moderate contusion SCI model at short-term (1 week post injury: Acute response) and long-term (6 weeks post-injury: Chronic response) time points.

Methods: PgP was synthesized and characterized as previously reported[2]. Rm was loaded into PgP using the solvent evaporation method and the loading efficiency was measured by HPLC as previously reported[1]. SD rats (male, 200-250 g) were deeply anesthetized, and a moderate contusion injury model was generated by impacting at T9 spinal cord with a force of 200 kdyn (IH-0400 impactor, PSI). For the catheterization, the spinal cord at lumbar level (L4-5) was exposed and the catheter (32 G, ReCathCo, LLC) was inserted through a hole made in the dura. To evaluate the effect of repeat administration of Rm-PgP, rats were divided into 4 groups; sham, untreated SCI (saline, 40 µl), Rm-PgP (20 µg Rm, 40 µl) single injection (Rm-PgP-S), Rm-PgP (20 µg Rm, 40 µl) repeat injection at 0, 2, and 4 DPI (Rm-PgP-R). Rm-PgP or saline was injected using microinjection pump at 2 µl/min. To analyze the effect of Rm-PgP on cAMP level, rats were sacrificed for harvesting fresh spinal cord at 1 week after injury and cAMP level was measured using a Mouse/Rat cAMP Parameter Assay Kit (R&D Systems). To evaluate the effect of Rm-PgP treatment on the inflammatory response and neuronal survival, histological analysis was performed at 1 week post-injury. Animals were sacrificed via cardiac perfusion using saline followed by 4% PFA under deep anesthesia. Spinal cords were retrieved, fixed, frozen, and cryo-

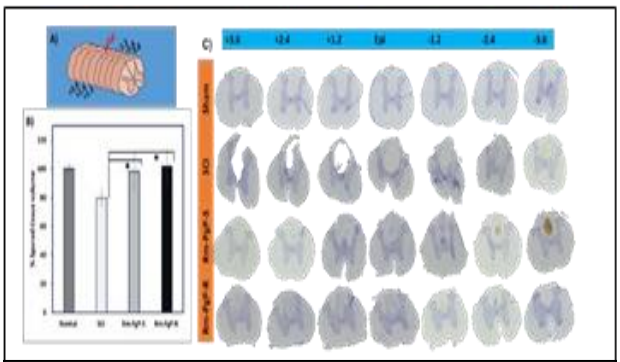

Figure 1. Spared tissue volume by Rm-PgP single and repeat injection at 1week post-injury in a rat moderate contusion SCI model. A) Diagram of section position (+: Rostral, Epi: Epicenter, -: caudal), B) % spared tissue volume. Data presented mean ± STD (n=7/group), and C) representative images of nissl stained transverse sections.Original magnification:100*.

sectioned transversely at 20 μm thickness. Nissl staining was performed to measure cavity size and IHC conducted with antibodies specific for ED1 and Arg1 to identify M1 and M2 macrophages, NeuN and GFAP for neuronal nuclei and reactive astrocytes. The stained sections were imaged by All-in-one inverted fluorescence microscope (Keyence BZ-X810) and the images were used for lesion area and positive cell counting using ImageJ software. The effect of Rm-PgP treatment on motor functional recovery and neuropathic pain was evaluated using Basso Bettie and Brenahan (BBB) scoring system and von Frey test for up to 6 weeks.

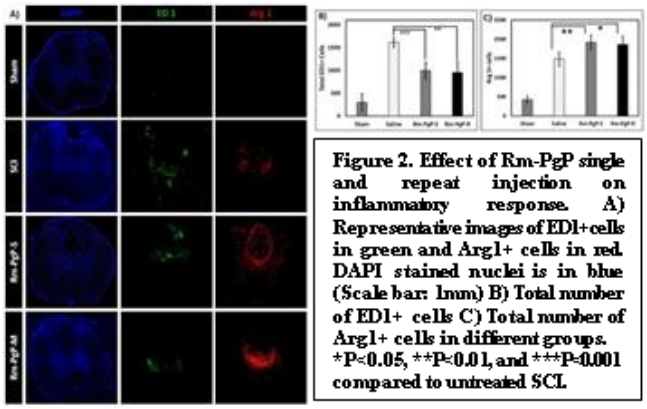

Figure 2. Effect of Rm-PgP single and repeat injection on inflammatory response. A) Representative images of ED1+cells in green and Arg1+ cells in red. DAPI stained nuclei is in blue (Scale bar: 1mm) B) Total number of ED1+ cells C) Total number of Arg1+ cells in different groups. *P<0.05, **P<0.01, and ***P<0.001 compared to untreated SCI.

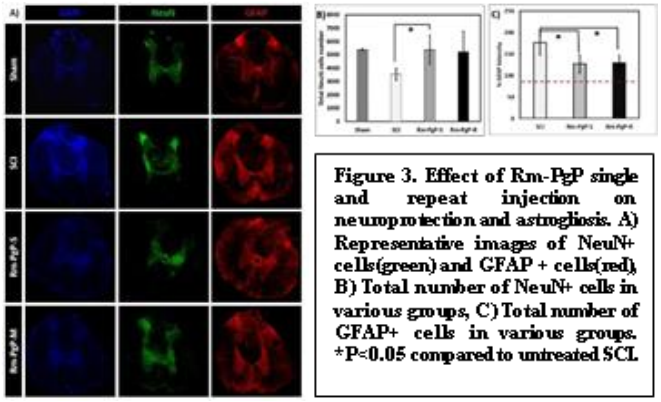

Figure 3. Effect of Rm-PgP single and repeat injection on neuroprotection and astrogliosis. A) Representative images of NeuN+ cells(green) and GFAP + cells(red), B) Total number of NeuN+ cells in various groups, C) Total number of GFAP+ cells in various groups. *P<0.05 compared to untreated SCI.

Results: We observed that cAMP level in Rm-PgP single and multiple injected groups was not significantly different with that in sham group, while cAMP level in untreated SCI group was significantly lower that in sham group at 7 DPI. Both single and repeat Rm-PgP injection significantly reduced the lesion volume compared to untreated SCI (Fig. 1). We also observed that both single and repeat Rm-PgP treatment significantly reduced the number of ED1+macrophages and increased Arg1+ cells (Fig. 2). The number of NeuN+ cells was significantly increased, indicating an increase in neuron cell survival following Rm-PgP treatment. Decreased GFAP fluorescence intensity indicated that astrogliosis was decreased by Rm-PgP treatment (Fig. 3). We observed that BBB scores of both Rm-PgP single and repeat injected animals were significantly higher than those of untreated SCI animals beginning at Day 5 and continuing throughout all subsequent time points. We observed that Rm-PgP single injection group showed slightly higher BBB score compared to Rm-PgP repeat injection group even though they were not significantly different. The BBB scores of Rm-PgP single treated group were not significantly different with sham group at 6 weeks time point. For neuropathic pain, we observed that pain level in both Rm-PgP single and multiple injection group was significantly lower than that in untreated SCI group (Fig. 4).

Conclusion: These results suggest that both single and repeated Rm-PgP via intrathecal injection, which is a less invasive and more clinically relevant administration route, significantly improve motor functional recovery and reduce neuropathic pain development.

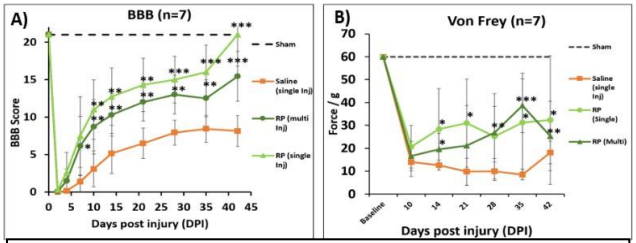

Figure 4. Effect of cAMP restoration after Rm-PgP single and repeatinjection on motor function and neuropathic pain after contusion SCI.
***P<0.05, **P<0.01, and ***P<0.001 compared to untreated SCI.**

Acknowledgement: This work was supported by NINDS of the NIH [grant number 5R01 NS111037-02].

References: 1.Macks et al., Journal of Neurotrauma, 35, 582–592 (2018), 2. Gwak *et al*., *Acta Biomater.* 35:98-108 (2016)

Preclinical Safety and Efficacy of Juvenile Cartilage-Derived Chondrocyte Sheets for Treating Focal Chondral Injury

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Purpose: Cartilage lacks an innate repair capability after traumatic injury. As such, focal cartilage defects have been implicated as a potential initiator of early osteoarthritic disease, and early treatment of chondral defects may prevent or delay the development of osteoarthritis and improve patient quality of life. Cell sheet technology using temperature responsive cultureware allows production of confluently cultured cells as sheets. Harvested sheets are readily patched on tissue lesions and adhere without suture, fibrin glue or other adhesion techniques. Autologous chondrocyte sheet transplantation has demonstrated safety and clinical efficacy in a study with combined anterior cruciate ligament reconstruction or high tibial osteotomy and microfracture. Juvenile cartilage-derived chondrocyte (JCC) has been identified as a quality source for neocartilage regeneration due to its immune tolerant acceptability, proliferative activity, and maintenance of cartilage-specific matrix proteins *in vitro*, as compared to adult cartilage-derived chondrocytes. Its availability also allows chondral defect treatment in a single-stage surgical procedure. This study aims to demonstrate the preclinical safety and efficacy of human polydactyly-sourced JCC sheets *in vitro* and *in vivo* using an athymic nude rat focal chondral defect model.

Methods: Cartilage from the phalanx and metacarpal bones of amputated polydactylous fingers and toes from 12 patients (24.8±17.0 months old) was used. Isolated chondrocytes with collagenase were expanded in chondrocyte culture medium (DMEM-F12 containing 1% antibiotic-antimycotic and 20% fetal bovine serum). Medium was replaced with chondrocyte medium supplemented with 100 μg/mL L-ascorbic acid phosphate magnesium salt n-hydrate at the first medium change. Expanded cells were collected by TrypLE Select and cryopreserved in STEM-CELLBANKER GMP grade at the end of P0. Cell sheets were prepared from thawed cryopreserved juvenile chondrocytes after one passage with a cell density of 10,000 cells/cm^2 on temperature responsive cell culture inserts. After 2 weeks of culture, cell sheets were harvested and characterized on cell viability, total cell number, and molecular markers with flow cytometry and immunohistochemistry. A focal chondral defect (diameter 2 mm; depth 200-350 μm) on nude

rats of 7 weeks old was created on the patellar groove of the femur using an electric grinder. Defect depth was controlled by the procedure under a surgical stereo zoom microscope with repeated depth measurement to achieve minimal damage on subchondral bones. For treatment group, JCC sheets were transplanted to each surgical knee defect after defect creation. Weight distribution in rats was demonstrated with Incapacitance Tester. Animals were sacrificed after 4, 8, 12, and 24 weeks for further histological evaluations.

Results: Polydactyly cartilage samples were characterized as hyaline cartilage using safranin-O staining. The isolated chondrocytes exhibited a constant growth rate for over 10 passages and maintained *in vitro* chondrogenic potential. JCC sheets maintained high cell viability (98.0 ± 1.3%) and rich cell numbers in each sheet construct ($1.90 \pm 0.48 \times 10^6$ cells per sheet). After detachment, JCC sheets undergo a spontaneous, endogenous contraction resulting in a multi-cell thick sheet structure. The cell sheet stains positively for aggrecan with immunohistochemistry. Importantly, cells isolated from JCC sheets exhibit robust chondrogenic capacity in pellet cultures. Cell surface markers of the JCC consisting cells for leukocytes and vascular endothelial cells were negligible, indicating that the isolation and culture processes are free of contaminants. All cells in sheets expressed mesenchymal cell markers, CD44, CD90 and CD81. Doubling time of cell growth was stable in serial subculture up to P13, which suggests that chondrocytes in sheets are unlikely to transform to infinitely proliferative cells in a further scaled production process. After focal chondral injury in nude rat model, fibrotic pannus indicative of failure to spontaneously regenerate cartilage tissue was observed in the defect only group at all time points (4, 8, 12, and 24 weeks). In contrast to the defect only group, complete fill regenerated white cartilage occurred in defect areas of the JCC sheet treatment group at all time points. Histological analysis showed the samples of defect only group are safranin-O negative, whereas the JCC sheet treatment group exhibited safranin-O positive, thick hyaline neocartilage at all time points (4, 8, 12, and 24 weeks) with a stably integrated interface with host tissue confirmed with histological analysis. In addition, formation of lacuna structure was observed at all time points, suggesting that mature cartilage was formed in defect areas. Importantly, the newly formed cartilage did not display tumorigenic growth in cartilage and other tissue over 6 months, supporting JCC sheet phenotypic stability and safety *in situ*. Immunohistochemical analysis showed that pannus tissue in the defect only group showed neither aggrecan (ACAN) nor type 2 collagen (COL2) expression, but strong type 1 collagen (COL1) expression. Regenerated neocartilage in the JCC sheet treatment group demonstrated expression of ACAN and COL2 with limited expression of COL1 localized to neocartilage surfaces. Origin of the regenerated tissue was determined as human cells by using human-specific vimentin staining. Interestingly, COL2 was observed at the periphery of human-vimentin positive cells and adjacent interstitial matrix. The cell sheet transplanted group showed rapid recovery to reach equal weight distribution on each leg after 3 weeks, while the defect only group sustained low weight distribution on the injured leg for over 6 weeks, indicating that regenerated cartilage tissue alleviates pain caused by the focal defect.

Conclusions: This study demonstrates the quality and safety of JCC sheets from a sustainable tissue supply and *in vivo* hyaline cartilage formation with JCC sheets in a nude rat focal chondral defect model.

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