THE 9TH BIENNIAL MEETING OF THE ISRAELI CHAPTER OF THE CONTROLLED RELEASE SOCIETY

NEW TRENDS AND TECHNOLOGIES IN DRUG DELIVERY AND IN CONTROLLED-RELEASE BASED CONSUMER PRODUCTS

September 9-11, 2014

Hacienda Forestview Hotel, Maalot, Israel

ORGANIZING COMMITTEE

Prof. Ronit Satchi-Fainaro, Tel-Aviv University - ICRS President

Dr. Ayelet David, Ben-Gurion University of the Negev

Prof. Boaz Tirosh, The Hebrew University of Jerusalem

Prof. Avi Domb, The Hebrew University of Jerusalem

Prof. Smadar Cohen, Ben-Gurion University of the Negev

Prof. Chezy Barenholtz**,** The Hebrew University of Jerusalem

Dr. Avi Schroeder, Technion – Israel Institute of Technology

Prof. Rimona Margalit, Tel-Aviv University

Prof. Dan Peer, Tel-Aviv University

Prof. Shulamit Levenberg, Technion – Israel Institute of Technology

Prof. Rosa Azhari, Ort Braude

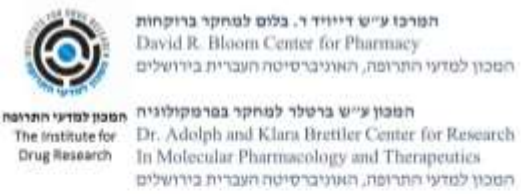
Prof. Marcelle Machluf, Technion – Israel Institute of Technology

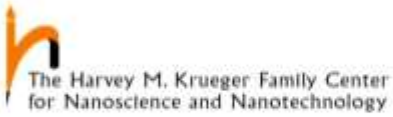
Dr. Raphael Nudelman, Teva Pharmaceutical Industries

Dr. Anat Eldar-Boock, Tel-Aviv University

Dr. Dikla Ben-Shushan, Tel-Aviv University

SPONSORS





The ICRS also wishes to thank **Mori Arkin, Avi Domb and Chezi (Chai) David Fund by Prof. Smadar Cohen** for their generous contributions

PROGRAM

Tuesday –September 9, 2014

From 8:00 **BUS TRANSPORTATION FROM** the Technion, Tel Aviv University, Hebrew University of Jerusalem- Hadassah Ein Karem, Ben-Gurion University of the Negev

12:30 - 14:00 **REGISTRATION AND POSTERS SETUP**

13:00 **LUNCH**

14:00 - 14:15 **WELCOME AND INTRODUCTORY REMARKS**
Ronit Satchi-Fainaro, ICRS President

14:15 – 16:20 **SESSION 1**
NANOMEDICINES IN CANCER TREATMENT
Chairpersons: *Ehud Gazit and Ronit Satchi-Fainaro*

14:15 – 15:00 **KEYNOTE PRESENTATION**
Prof. Henry Brem (Johns Hopkins University) – "Targeted Brain Tumor Therapy"

15:00 – 15:20 **Avi Schroeder** (Technion) – "Targeted Drug Delivery and Personalized Medicine"

15:20 – 15:40 **Ester Segal** (Technion) – ""Nanostructured Porous Silicon: A Tunable Platform for Delivery of Anticancer Therapeutics"

15:40 – 16:00 **Osnat Ashur-Fabian** (Meir Medical Center) – "Novel Application of αvβ3 Integrin for Targeted Therapy in Cancer"

16:00 – 16:20 **Vered Padler-Karavani** (TAU) – "Targeting a Non-Human Sugar for Cancer Theranostics"

16:20 - 17:00 **COFFEE BREAK**

17:00 - 18:00 **SESSION 2**
ADVANCED FORMULATIONS FOR TARGETING SITES OF INFLAMMATION
Chairpersons: *Yechezkel Barenholz and Ayelet David*

17:00 – 17:20 **Rimona Margalit** (TAU) – "Inhalational Anti-Oxidant and Anti-Inflammatory Treatment of Mice Exposed to Toxic Industrial Chemicals Applying Targeted Drug-Carrier Formulations"

17:20 – 17:40 **Jonathan Leor** (Sheba Medical Center) – "Targeting Inflammation in Cardiovascular Disease"

17:40 – 18:00 **Dan Peer** (TAU) – "To Target or Not to Target? Selectivity Hurdles in Targeted Nanomedicines for Leukocytes-Implicated Diseases"

18:00 - 19:00 ***Short presentations*** *(15 x 3 min) of selected posters from submitted abstracts*
Chairpersons: *Nissim Garti and Vered Padler-Karavani*
(1) Alona Goldstein (HUJI) – "Targeted Gold Nanoparticles in the Treatment of Psoriasis"
(2) Stasia Krishtul (Technion) – "Targeted Therapy of Metastatic Cancer Using Cell Derived Nano-Ghosts"
(3) Nitzan Marelly (BGU) – "Development of Modified Starch Based Complexes for Pi3P Delivery in Order to Overcome Insulin Resistance"
(4) Hemda Baabur-Cohen (TAU) – "Anticancer Polymeric Nanomedicine Bearing Synergistic Drug Combination"
(5) Nurit Becker (TAU) – "Recombinant Immunotoxins for the Treatment of Hematological Malignancies"
(6) Tal Berman (HUJI) – "LC-100, a Novel Pegylated Liposomal Doxorubicin Nano-Drug Superior to Doxil®: Design in vivo and in vitro characterization"
(7) Nitzan Letko (Technion) – "Targeting Nano-Ghosts, Cell-Derived Drug-Carriers, for the Treatment of Inflamed Tissues"
(8) Liat Frid (TAU) – "Design, Synthesis and Utillization of Internally Functionalized Peg-Dendron Hybrids as Novel Drug Delivery Platforms"
(9) Shiran Ferber (TAU) – "Reciprocal Dormancy-Promoting Nanomedicine Altering EGFR and TSP-1 for the Management of Glioblastoma"
(10) Zvi Yaari (Technion) – "Theranostic Barcoded Nanoparticles"
(11) Yael Lupu-Haaber (Technion) – "Interactions of Nano-Ghosts Derived from Mesenchymal Stem Cell with the Tumor Niche"
(12) Gil Aizik (HUJI) – "Imaging of Vascular-Injured Tissue by Liposomal Quantum-Dots"
(13) Ilan Winkler (HUJI) – "A Novel Liposomal Bupivacaine Formulation to Produce Ultralong-Acting Analgesia"
(14) Dafna Knani (Braude College) – "Simulation of Bilayer Systems Based on Neutral and Cationic Lipids in Combination with Fluorescent Lipids"
(15) David Stepensky (BGU) – "Quantitative Analysis of Drug Delivery to the Brain via Nasal Route"

20:00 **COCKTAIL RECEPTION AND POSTER SESSION**

Wednesday – September 10, 2014

8:00 - 8:30 **ADMINISTRATIVE SESSION**: General Assembly of ICRS
ICRS elections!

8:30 - 10:15 **SESSION 3**
INNOVATIVE DELIVERY VEHICLES (1)
Chairpersons: *Avi Domb and Joseph Kost*

8:30 – 9:15 **KEYNOTE PRESENTATION**

Prof. Martyn Davies (Nottingham University) – "Nanotechnology on the Pharmaceutical Sciences: From Lab to Industry"

9:15 – 9:35 **Nissim Garti** (HUJI) – "Lipid -Based Molecular Architectures for Drug Delivery"

9:35 – 9:55 **Ehud Gazit** (TAU) – "Self-Assembly and Manipulation at the Nano-Scale for the Development of Novel Drugs, Diagnostics and Biomaterials"

9:55 – 10:15 **Itai Benhar** (TAU) – "Design Principles for Bispecific Iggs- the H-L Pairing Challenge"

10:15 - 10:45 **COFFEE BREAK**

10:45 - 12:05 **SESSION 4**
INNOVATIVE DELIVERY VEHICLES (2)
Chairpersons: *Doron Shabat and Dan Peer*

10:45 – 11:05 **Marcelle Machluf** (Technion) – "Nano Ghost Delivery Systems Are Spooking Cancer"

11:05 – 11:25 **Boaz Mizrahi** (Technion) – "Short, Multi-Armed Pre-Polymers"

11:25 – 11:45 **Roey Amir** (TAU) – "Smart Micellar Nanocarriers"

11:45 - 12:05 **Joseph Kost** (BGU) – "Stimuli Responisive Systems in Drug and Gene Delivery Applications"

12:05 - 12:45 **SESSION 5**
OVERCOMING BARRIERS IN THE GI TRACT
Chairpersons: *Avri Rubinstein and Roy Weinstain*

12:05 – 12:25 **Avi Domb** (HUJI) – "Enhanced Oral Bioavailability of Hydrophobic Drugs"

12:25 – 12:45 **Ofra Benny** (HUJI) – "Polymer Nanomicelles as a Platform for Oral Drug Delivery in Cancer"

12:45 - 14:45 **LUNCH FOR ALL PARTICIPANTS AND POSTER VIEWING**

14:45 – 16:45 **SESSION 6**
TARGETING AND DELIVERY IN REGENERATIVE AND PERSONALIZED MEDICINE
Chairpersons: *Smadar Cohen and Rosa Azhari*

14:45 - 15:05 **Shulamit Levenberg** (Technion) – "Localized Stimulation of Differentiation in a Human Embryoid Body Model using Microsphere-Coupled Factors"

15:05 - 15:25 **Alejandro Sosnik** (Technion) – "Do Not Forget the Forgotten Diseases…and Patients"

15:25 - 15:45 **Tal Dvir** (TAU) – "Advanced Biomaterials for Cardiac Tissue Engineering"

15:45 - 16:05 **Alberto Gabizon** (Shaare Zedek Medical Center) – "Liposomal Delivery of a Lipophilic Anti-Cancer Prodrug: From Bench to Bedside"

16:05 - 16:25 **Michael Meijler** (BGU) – "Effects of Slow Release of Quorum Sensing Inhibitors on Virulence in *Pseudomonas aeruginosa*"

16:25 - 16:45 **Ruth Gabizon** (HUJI) – "Novel Pomegranate Oil Nano-Emulsions for the Prevention and Treatment of Neurodegenerative Diseases: The Case of Genetic Cjd"

16:45 – 17:15 **COFFEE BREAK**

17:15 - 18:15 **SESSION 7**

IP DISCUSSION PANEL

"Intellectual Property with a View to the Future of Humanity's Developmental Challenges: New Paradigm?"

Moderator: *Dr. Ilan Cohn, Senior Partner, Reinhold Cohn Group*

Panel Members: Martin Gerstel (Compugen, Mada BioScience, and Evogene), Ehud Gazit (TAU), Assaf Barnea (iNSPIRE incubator formed by Philips and Teva).

18:15 – 19:00 ***Short presentations*** *(15 x 3 min) of selected posters from submitted abstracts*

Chairpersons: *Ofra Benny and Dafna Knani*

(1) Daphne Weihs (Technion) – "Time-Dependent Endocytotic Uptake, Localization, and Encapsulation of Sub-Micron Particles Depends on Cell Malignancy"

(2) Rachel Blau (TAU) – "Non-Invasive Intravital Monitoring of Drug Release from Novel Polymeric Theranostic nomedicines"

(3) Nizan Cauzmer (TAU) – "Gagomers as Cisplatin Carriers for Therapeutic Intervention in Lung Cancer"

(4) Yael Efraim (Technion) – "Injectable Porcine Cardiac Extracellular Matrix as a Cell latform for Myocardial Regeneration"

(5) Moran Golan (BGU) – "CD44-Targeted Polyion Complexes for siRNA Delivery"

(6) Assaf Zinger (Technion) – "Bio-Surgery"

(7) Anat Eldar-Boock (TAU) – "Neoadjuvant Treatment for Prevention of Breast Cancer Metastasis Development: αvβ3 Integrin-Targeted PGA-Paclitaxel Nanocunjugate"

(8) Assaf Harnoy (TAU) – "Enzyme-Responsive Amphiphilic PEG-Dendron Hybrids and Their Assembly into Smart Micellar Nanocarriers"

(9) Chaya Mazouz (HUJI) – "Regulatotry Highlights of the Long and Complicated Path from the Bench to the Market"

(10) Limor Kaneti (Technion) – "Cell Derived Nano-Ghosts as a New Gene Delivery System for Cancer Therapy"

(11) Emma Portnoy (HUJI) – "Tracking Inflammation in the Epileptogenic Brain Tissue in the Rat by Multifunctional Nanoparticles"

(12) Nour Zoaby (Technion) – "Nanoswimmers for Targeted Drug Delivery"

(13) Almog Bitton (TAU) – "PfSUB1-Activated Immunozymoxins for Treating Malaria"

(14) Ravit Chen (Institute for Biological Research) – "Fluorescent Polystyrene/ polystyrylbisphosphonate Core Shell Microspheres – A Potential Bone Cement Component"

(15) Michal Shevach (TAU) – "Nanoengineering Gold Nanoparticle - Composite Scaffolds for Cardiac Tissue Engineering"

19:00 - 19:30 **POSTER VIEWING**

20:30 **GALA DINNER**

- Presentation of the ICRS Prize for Outstanding Achievements in Controlled Release
- Presentation of the 2014 Best Student Poster Awards: First prize, Second prize and three Third prizes.

Thursday –September 11, 2014

Check-out at 9:00 am

9:00 - 10:50 **SESSION 8**

ADVANCES IN BIOMEDICAL IMAGING

Chairpersons: *David Stepensky and Avi Schroeder*

9:00 – 9:30 **Yechezkel Barenholz** (HUJI) – "Relevant Physicochemical and Biophysical Characteristics as the Foundation of the Development and Approval of Nano-Drugs"

9:30 – 9:50 **Doron Shabat** (TAU) – "Donor-Two-Acceptors Dye Design - a Distinct Gateway to NIR Fluorescent Probes"

9:50 – 10:10 **Galia Blum** (HUJI) – "Characterization of Cathepsin Activity in Vulnerable Atherosclerotic Plaque Macrophages and Applications in Imaging and Therapy"

10:10 – 10:30 **Roy Weinstain** (TAU) – "In Vivo Targeting of Hydrogen Peroxide by Activatable Cell-Penetrating Peptides"

10:30 – 10:50 **Yael Mardor** (Sheba Medical Center) – "The Application of Convection-Enhanced Drug Delivery for the Treatment of Brain Tumors – Advantages and Challenges"

10:50 - 11:30 **COFFEE BREAK**

11:30 - 13:30 **SESSION 9**

INTRACELLULAR DELIVERY OF DRUGS AND NUCLEIC ACIDS

Chairpersons: *Rimona Margalit and Roey Amir*

11:30 – 11:50 **Eylon Yavin** (HUJI) – "Peptide Nucleic Acids (PNA) as Anti-Malaria Agents"

11:50 – 12:10 **Smadar Cohen** (BGU) – "Anionic Polyplexes for siRNA Delivery"

12:10 – 12:30 **Jean-Paul Lellouche** (BIU) – "Gamma-Maghemite-Polymer Hybrid Nanocomposites for siRNA/MicroRNA Delivery/Gene Silencing Applications: Innovative Chemical Strategies for Toxicity Control"

12:30 – 12:50 **Ayelet David** (BGU) – "CD44-Targeted Systems for Intracellular Delivery of Chemotherapeutic Drugs and Small Interfering RNA (siRNA)"

12:50 – 13:10 **Simon Benita** (HUJI) – "Nano Delivery Systems of Hydrophilic Biomacromolecules for Improved Therapy"

13:10 – 13:30 **Amotz Shemi** (Silenseed) – "RNAi-Based Therapy for Solid Tumors - Results from Phase I/IIb in Pancreatic Cancer"

13:30 - 14:00 **CONCLUDING REMARKS**

Ronit Satchi-Fainaro, ICRS President

14:30 **BUS TRANSPORTATION BACK TO** the Technion, Tel Aviv University, Hebrew University of Jerusalem- Hadassah Ein Karem, Ben-Gurion University of the Negev.

ABSTRACTS

TARGETED BRAIN TUMOR THERAPY

Henry Brem and Betty Tyler

Departments of Neurosurgery, Oncology, Ophthalmology, and Biomedical Engineering Johns Hopkins University, Baltimore, Maryland

The technology of local delivery is being utilized to target multiple pathways for brain tumor therapy. Through the combination of Gliadel, radiation therapy and oral temozolomide (TMZ), median patient survival has increased from 9 to 21 months. To further increase survival, we are exploring the local delivery of TMZ and have shown that in animal models we can achieve 37.5% long term survival. This benefit dramatically increases when combined with radiation therapy. Polymer delivery of anti-proliferative agents such as the microtubule stabilizer paclitaxel, the topoisomerase I inhibitor camptothecin, and the mTOR inhibitor rapamycin show promise in preclinical studies. Tumor angiogenesis is targeted with treatment of anti-angiogenic agents, such as bevacizumab and Fc-endostatin. Local delivery of the anti-glutaminergic agent riluzole protects normal brain and decreases tumor invasion. Vaccines have shown great promise with local polymer delivery. Anti-glycolytic agents are being explored through local delivery for their effect on tumor inhibition. Our delivery of various mRNA and siRNAs through biodegradable poly(beta-amino ester) (PbAE) nanoparticles is showing promising efficacy in our rodent and human glioma lines in vivo. We are using ultrasound to increase the distribution of pectin-based nanoparticles and to enhance the permeability of the cell membrane to allow for increased therapeutic efficacy. A logical evolution of local controlled release is the creation of devices that release multiple agents in multiphasic patterns to optimize therapeutic regimens or more accurately recapitulate the physiologic norm. This was addressed with the development of biodegradable poly (L-lactic acid) multi-well passive microchips with poly (D,L-lactic-co-glycolic acid) membranes. Development of this therapy heralds a new approach to brain therapeutic research, offering the ability to circumvent physiologic barriers and effectively deliver a multitude of novel agents. Novel imaging methods, including optical coherence tomography and laser speckle contrast imaging are in development to better characterize angiogenesis and subsequent tumor growth as well as assess microvascular morphology and the blood flow of individual microvessels. Through the combination of these approaches we are hoping to deliver more potent biological agents to continue to improve the survival and quality of life for cancer patients.

TARGETED DRUG DELIVERY AND PERSONALIZED MEDICINE

Avi Schroeder
Chemical Engineering, Technion - Israel Institute of Technology, Israel

The field of medicine is taking its first steps towards patient-specific care. Our research is aimed at tailoring treatments to address each person's individualized needs and unique disease presentation. Specifically, we are developing nanoparticles that target diseased tissue, where they perform a programmed therapeutic task. These systems utilize molecular-machines to improve efficacy and reduce side effects.

Two examples will be described: the first involves a nanoscale theranostic system for predicting the therapeutic potency of drugs against metastatic cancer. The system provides patient-specific drug activity data with single-cell resolution. The system makes use of barcoded nanoparticles to differentiate between the therapeutic effects different drugs have on the tumor microenvironment.

The second system makes use of enzymes, loaded into a biodegradable chip, to perform a therapeutic task – surgery with molecular precision. Collagenase is an enzyme that cleaves collagen, but not other tissues. This enzyme was loaded into the biodegradable chip and placed in the periodontal pocket. Once the collagenase releases from the chip, collagen fibers that connect between the teeth and the underlying bone are relaxed, thereby enabling enhanced orthodontic corrective motion and reducing pain. This new field is termed BioSurgery.

The efficacy of both systems was demonstrated in vitro and in vivo using relevant pre-clinical models.

The clinical implications of these approaches will be discussed.

NANOSTRUCTURED POROUS SILICON: A TUNABLE PLATFORM FOR DELIVERY OF ANTICANCER THERAPEUTICS

Adi Balter[1], Zohar Shatsberg[2], Margarita Beckerman[2], Natalie Artzi[2], **Ester Segal**[1]
[1]*Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[2]*Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, USA*

Local drug delivery systems are increasingly under development as systemic drug administration is associated with detrimental side effects and low drug bioavailability. Nanostructured porous Silicon (PSi) emerges as a promising platform for drug delivery owing to its high biocompatibility, degradability, high surface area and internal volume available for drug loading. The potential impact of PSi on future healthcare is evident by the current assessment of various PSi devices for medical applications in clinical trials.

Our work focuses on the design and synthesis of PSi matrices as carriers for different antineoplastic drugs, tailoring their nanostructure and surface properties to exhibit a desired release profile. The resulting PSi carriers demonstrate high loading efficacy of different drugs and profound cytotoxicity towards MDA-MB-231 cells *in vitro*. When progressing to *in vivo* models, we revealed that correlation between *in vitro* and *in vivo* behavior of PSi persists only under specific conditions that mimic local oxidative stress manifested by the tumor microenvironment. Under these conditions PSi erosion is enhanced compared to healthy state. Using our model system, we identify determinant factors that modulate material erosion and drug release (doxorubicin is used as a model drug) to begin to unravel the importance of the physiological microenvironment in determining device performance and therapeutic capacity.

NOVEL APPLICATION OF αvβ3 INTEGRIN FOR TARGETED THERAPY IN CANCER

Osnat Ashur-Fabian

Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel-Aviv University

Translational Hemato-Oncology, Meir Medical Center

αvβ3 integrin is a cell surface receptor that plays a pivotal role in cancer pathogenesis and is intensively studied. It interacts with proteins of the extra cellular matrix (ECM) through an RGD (Arg-Gly-Asp) recognition site. αvβ3 integrin is over expressed in all tumor vascular cells and in an array of cancer types, in correlation with tumor progression. Hence, blocking αvβ3 action is an attractive and rational target for cancer therapy.

Integrin blockers are mainly based on RGD-containing peptides or functional anti αvβ3 antibodies. However, clinical results are not yet sufficient and such inhibitors can paradoxically enhance angiogenesis, tumor aggressiveness and invasiveness. Therefore, it would be sensible to identify new sites upon this integrin that can be effectively targeted without promoting tumor pathogenesis.

In the past years, αvβ3 integrin was shown to interact with a structurally diverse range of non-RGD amino acid sequences and small molecules. This implies that this integrin possesses non-RGD binding sites.

Recently, upon the αvβ3 integrin, at close proximity to the RGD site, an independent novel non-RGD binding site for thyroid hormones was discovered. Following binding of the hormones to this site, proliferative and angiogenic activities are exerted through the MAPK/ Pi3K/Hif1α pathways. Several potential antagonists based on non-RGD mimics, including tetraiodothyroacetic acid (tetrac), nano-formulated tetrac (tetrac-NP) and triiodothyroacetic acid (triac), are under active research. These antagonists were shown to effectively inhibit cancer cell division, migration and angiogenesis in vitro and in vivo.

TARGETING A NON-HUMAN SUGAR FOR CANCER THERANOSTICS

Vered Padler-Karavani
Cell Research and Immunology, Tel Aviv University

All living cells are covered with a dense layer of sugars (glycans). They are found either as free forms or covalently attached to proteins or lipids (glycoconjugates). Several types of glycan families are represented on the cell surface. Altered cell surface glycosylation is a common feature of cancer cells that result from abnormal expression of glycosyltransferases. Sialic acids (Sias) are 9-carbon backbone acidic sugars found at the outermost positions of glycan chains on various glycoproteins and glycolipids on vertebrate cell surfaces and secreted glycans. Variations in Sias expression patterns are especially frequent on cancer cells and often correlate with advanced stage, progression and/or metastasis. The two major Sia forms in mammals are *N*-acetylneuraminic acid (Neu5Ac) and its hydroxylated form, *N*-glycolylneuraminic acid (Neu5Gc). Humans cannot synthesize Neu5Gc due to a specific inactivation of *CMAH* encoding CMP-Neu5Ac hydroxylase. Despite that, this non-human Sia metabolically incorporates in human cells as 'self', apparently originating from dietary Neu5Gc-rich foods (e.g., red meat). Neu5Gc is present at low levels on cell surfaces of human epithelia and endothelia, but especially accumulates on carcinomas. Consequently, it is recognized as foreign by the human immune system and result in broad anti-Neu5Gc antibodies response. Low levels of these antibodies promote weak chronic inflammation facilitating tumor progression that can be suppressed by anti-inflammatory drugs. Likewise, anti-Neu5Gc antibodies could potentially play a role in vascular inflammation disease states such as atherosclerosis. However, at higher concentrations, these antibodies suppress growth of Neu5Gc-expressing tumors. Some of these antibodies are also potential immunotherapeutics as they could promote complement- or antibody-dependent cellular cytotoxicity (CDC/ADCC) on related human cancer cells. Using a newly developed sialoglycan-microarray, a unique anti-Neu5Gc IgG was discovered as a novel carcinoma biomarker. Altogether, these findings highlight the immune recognition of incorporated dietary non-human sugar in humans as a novel target for cancer theranostics.

INHALATIONAL ANTI-OXIDANT AND ANTI-INFLAMMATORY TREATMENT OF MICE EXPOSED TO TOXIC INDUSTRIAL CHEMICALS APPLYING TARGETED DRUG-CARRIER FORMULATIONS

Rimona Margalit[1], Ilia Rivkin[1], Yifat Galnoy-Glucksam[1], Inbar Elron-Gross[1], Amichay Afriat[2], Arik Eisenkraft[2]

[1]*Biochemistry and Molecular Biology, Tel Aviv University, Israel*

[2]*2 NBC Protection Division, Israel Ministry of Defense, Israel*

Control and treatment of respiratory damage caused by exposure - accidental or deliberate - to toxic industrial chemicals (TICs), is an unmet need due to: (1) the time span from injury to treatment initiation and (2) poor efficacy and safety of currently-available (free) drugs.

Our strategy to address this unmet need, especially in mass casualty events, was to: formulate the drugs in a targeted carrier; administer the formulations in an aerosol form directly to the injured airways and lungs; initiate treatment at the disaster area, having the formulations and portable inhalation devices in the vehicles of the first responders.

Three drug-carrier formulations, using hyaluronan liposomes as the targeted carrier, were prepared, encapsulating dexamethasone (Dex), N-acetyl cysteine (NAC), or both drugs in the same carrier. To obtain proof of concept in mice, chlorine was used as the TIC. Survival, behavior and weight changes of BALB/c mice exposed to - air alone (control), chlorine alone (300 ppm in air/30 minutes) or chlorine followed by treatment - were monitored over 2-3 weeks. The experiments were conducted in a specially-designed inhalation chamber.

Control mice gained weight continuously. All chlorine-exposed mice survived, but had a two-trend weight change: loss over the first two days, reversing thereafter to weight gain, yet both rate and level of the weight gain were significantly slower and smaller than those of the control mice. The chlorine-exposed mice given the inhalational liposomal-drug therapy also showed the two-trend weight change, but their rates and levels of weight gains were similar to those of the control mice, outperforming aerosols of free drugs. A single treatment sufficed and efficacy sequence of the liposomal drugs was NAC ~ NAC+Dex Dex. Based on this proof of concept, studies are now extended to additional TICs, and to biochemical markers of injury and recovery.

TARGETING INFLAMMATION IN CARDIOVASCULAR DISEASE

Jonathan Leor
Cardiovascular Research Institute, Te-Aviv University, Israel

One of the greatest challenges in modern cardiology is to control and optimize tissue regeneration and repair. Uncontrolled inflammation can aggravates the progression of atherosclerosis cardiovascular disease and adverse cardiac remodeling.

Nanomedicine is anticipated to improve early diagnosis, acute intervention and follow-up-therapy in cardiovascular disease. Nanomedical preparations can be instructed to target inflammatory processes in the infarcted myocardium and atherosclerotic vessels. For example, macrophage-targeted HA-liposomes with therapeutic molecules switch macrophages into a reparative phenotype, and improve cardiac remodeling and dysfunction after MI. Targeted carriers could deliver therapeutic molecules that stabilize the plaque and prevent rupture. Moreover, new treatments include intelligent nanobiomaterials with the ability to attract local stem cells or cultured cells to the site of injury, providing cell therapy that should improve heart function and decrease mortality of patients after MI.

The principles and strategies described here could be applicable to other cardiovascular diseases associated with macrophages and inflammation, such as atherosclerosis, myocarditis, and pulmonary hypertension.

TO TARGET OT NOT TO TARGET? SELECTIVITY HURDLES IN TARGETED NANOMEDICINES FOR LEUKOCYTES-IMPLICATED DISEASES.

Dan Peer
Cell Research and Immunology, Tel Aviv University, Israel

RNA interference (RNAi)-based approaches have greatly contributed to better understanding of gene expression and function *in vitro*. The capability to apply these strategies *in viv*o in order to validate the role of specific genes in normal or pathological conditions, and to induce therapeutic gene silencing, opened new avenues for utilizing RNAi as a novel therapeutic modality. However, the translation of RNAi from an effective genomic tool into a novel therapeutic modality has been hindered by the difficulty to deliver RNAi molecules into their target tissues by systemic administration, especially to hematopoietic cells. In this presentation, I will describe some of the challenges and opportunities in modulating leukocytes response using RNAi and discuss adverse effects such as immuno-toxicity. Special emphasize will be made on delivery strategies that target subsets of leukocytes such as the integrin-targeted and stabilized nanoparticles platform and the gagomers and I will detail examples from inflammatory bowel diseases, blood cancers and solid tumors.

Personalized nanomedicine has the power of combining nanomedicine with clinical and molecular biomarkers ("OMICS" data) achieving improve prognosis and disease management as well as individualized drug selection and dosage profiling to ensure maximum efficacy and safety. In this presentation, I will also detail aspects of personalized nanomedicine both from the drug and the carrier standpoint.

NANOTECHNOLOGY ON THE PHARMACEUTICAL SCIENCES: FROM LAB TO INDUSTRY

Martyn C. Davies

Professor of Biomedical Surface Chemistry, Laboratory of Biophysics and Surface Analysis. The School of Pharmacy, University of Nottingham, Nottingham NG7 2RD

Surface and Interfacial phenomena influence the function and performance of many pharmaceutical and biomedical systems. This presentation will provide an insight into how the surface chemistry, morphology and bioactivity of novel drug delivery systems and biomedical materials may be probed at the nanoscale using a suite of complimentary advanced biophysical analytical techniques. The potential of such techniques for high-resolution imaging, the measurement of molecular and inter-particulate forces, biorecognition events and determining interfacial chemical structure will be explored for systems for gene delivery, inhalation therapy and tissue engineering scaffolds. The talk will encourage a comprehensive approach for the characterisation of complex pharmaceutical systems and will highlight the recent developments in high throughput surface analysis that provide an rapid screening strategy that has been shown to be valuable in understanding biological interactions at tissue engineering scaffold interfaces for stem cell applications. The successful translation of these methodologies and technologies into the commercial field through the spin-out Molecular Profiles Ltd will be discussed. The company has exploited surface and interfacial techniques in pharmaceutical research and development to help identify and resolve problems, in assisting in the design of novel delivery systems and in helping to understand the in-life performance of materials within complex pharmaceutical systems.

LIPID -BASED MOLECULAR ARCHITECTURES FOR DRUG DELIVERY

Nisim Garti[1,2],

[1]*Department of Chemistry, Hebrew University, Israel*
[2]*Chemistry, Hebrew University*

In this presentation I will concentrate in bringing the major achievements derived from two families of vehicles for delivery of bioactives, nutraceuticals, antioxidants and drugs.

The first delivery vehicle was termed NSSL (Nano Size Self-assembled Liquid) and the second MLDS (Modified Lyotropic Delivery Systems).

Fluid discontinuous micellar cubic mesophases (QL vehicles) were made by incorporation of hydrotropes within the headgroups of the major lipophilic surfactants as spacers within the tails of the surfactants. In a similar way (different compositions) we managed to construct stable fluid modified reverse hexagonal mesophases (H^mII) at room temperature.

The length of the aqueous channels, the diameter of the tubes and the thickness of the oil phase were modified to accommodate high molecular weight guest molecules as solubilizates. Loading capacities and the interactions of various lipophilic as well as hydrophilic solubilizates (bioactive) with the surfactants interface were determined and characterized.

The vehicles were used to solubilize class IV bioactives both insoluble and non-bioavailable compounds (such as lycopene, CoQ10, pytosterols, lutein, DHA curcumin, vitamins D and E and various drugs) and macromolecules (such as proteins, enzymes, DNA and SiRNA).

Delivery profiles of various bioactives were studied across the guts membranes, skin (transdermal), ocular and transcellular.

Several types of specific molecules were ‘anchored’ on the interface of the vehicle such as ‘membrane recognition agents (PC)’, ‘penetrating agents’ and lipolysis enzymes to enable the structures to adhere to the membrane and open the tight junctions. The delivery patterns indicate enhanced penetration to the blood stream.

Protein-based drugs (growth hormones, cyclosporine, and desmopressin. calcitonin) were solubilized in very significant loads and delivered at will.

Multi step strategy was designed to deliver the drugs from the guts to the blood stream and into specific organs, cells using derivatized dendrimers and/or tannic acid.

SELF-ASSEMBLY AND MANIPULATION AT THE NANO-SCALE FOR THE DEVELOPMENT OF NOVEL DRUGS, DIAGNOSTICS AND BIOMATERIALS

Ehud Gazit
Department of Molecular Microbiology and Biotechnology, Department of Materials Science and Engineering, Tel Aviv University, Israel

The formation of amyloid fibrils is the hallmark of several diseases. In spite of grave clinical consequence, the mechanism of amyloid formation is not fully understood. We have suggested that aromatic interactions may provide energetic contribution, order and directionality in the self-association processes. Significant part of the activity in our lab is related to development of new therapeutic agents based on this notion. Our works lead to the discovery that the diphenylalanine recognition motif self-assembles into peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. We demonstrated that the nanostructures have unique chemical and physical properties including ultra-rigidity, semi-conductive, piezoelectric and optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. Finally, we had developed ways for depositing of the peptide nanostructures and their organization. We had use inkjet technology as well as vapour deposition to coat surface and from the peptide "nano-forests". We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct diffraction pattern and toxic properties.

REFERENCES:

- Reches, M., Gazit, E. (2006) Controlled Patterning of Aligned Self-Assembled Peptide Nanotubes. **Nature Nanotechnology** *1*, 195-200.
- Adler-Abramovich L. et al. (2009) Self-Assembled Arrays of Peptide Nanotubes by Vapour Deposition. **Nature Nanotechnology** *4*, 849-854.
- Adler-Abramovich, L. et al. (2012) Phenylalanine Assembly into Toxic Fibrils Suggests Amyloid Etiology in Phenylketonuria. **Nature Chem. Biol.** *8*, 701-706.
- Fichman, G., Gazit, E. (2014) Self-Assembly of Short Peptides to Form Hydrogels: Design of Building Blocks, Physical Properties and Technological Applications. **Acta Biomater.** *10*, 1671-1682.

DESIGN PRINCIPLES FOR BISPECIFIC IGGS - THE H-L PAIRING CHALLENGE

Lilach Vaks, Dana Greenfeld, Stav Dror, Leeron Shefet-Carasso, Rahely Hakim, Iris Alroy, **Itai Benhar**

Molecular Microbiology and Biotechnology, Tel-Aviv University, Israel

Bispecific antibodies (bsAbs) are antibodies with two binding sites directed at different antigens, enabling therapeutic strategies not possible with conventional monoclonal antibodies (mAbs). Since bispecific antibodies are regarded as promising therapeutic agents, several bispecific design modalities have been evaluated, but as most of them are small recombinant fragments, their utility is limited. In a nutshell, the field prefers IgGs.

Two criteria should be met to make bispecific IgGs; one is that each heavy chain will only pair with the heavy chain of the second specificity and that homodimerization be prevented. The second is that each heavy chain will only pair with the light chain of its own specificity and not with the light chain of the second specificity. The first solution to the first criterion (knobs into holes, KIH) was presented in 1996 by a group from Genentech. Additional solutions were presented more recently. However, until recently, no solutions for the second criterion that make it possible to produce a bispecific IgG by an expressing cell were suggested.
We present a solution for the second criterion; an engineered disulfide bond between the antibodies' variable domains. This approach termed disulfide stabilization, replaces the natural disulfide bond between the CH1 and CL domains. Moreover, the enginerred cysteins serve a dual purpose in facilitating disulfide pairing of the correct H-L pair and preventing the formation of incorrect H-L chain pairs. By combining KIH for heavy chains heterodimerization and disulfide stabilization for H-L chain pairing, we efficiently produced several bsAbs in bacteria and in mammalian cells.

In the presentation examples will be provided for the evaluation of some of these bsAbs and future directions of the study will be discussed.

NANO GHOST DELIVERY SYSTEMS ARE SPOOKING CANCER

Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion Israel Institute of Technology, Israel

The ultimate goal in cancer drug and gene delivery is a 'magic-bullet' that provides a versatile platform for site-specific targeting of multiple cancers, implemented in a clinically relevant and non-toxic design. We have designed a cancer delivery platform**,** which is based on unique cell-derived nano-ghosts (NGs) produced from whole cell membranes of mesenchymal stem cells (MSCs). MSC are well known for their natural targeting of multiple cancers and hypo-immunogenicity. Encompassing MSC surface proteins and armed with their unique targeting capabilities, the MSC-NGs may be loaded with various drugs and nucleic acids and can be selectively targeted against multiple cancers. Such a universal targeting platform can meet the three major prerequisites for an ideal delivery system: biocompatibility, long circulation time, and selectivity. It also represents a more clinically relevant approach than conventional delivery systems as it avoids the elaborate production and incorporation of targeting moieties into the delivery vehicle. This NG delivery platform can be used for a wide range of clinical applications, as well as contributing to our understanding of cancer progression and can readily be extended to cancer imaging and diagnostics, paving the way to possible treatments of other diseases.

SHORT, MULTI-ARMED PRE-POLYMERS

Boaz Mizrahi
Faculty of Biotechnology and Food Engineering, Technion, Israel

While high molecular weight pre-polymers (monomers) are receiving significant attention in polymer science, the development of low molecular weight monomers, particularly multi-armed (star shaped) monomers remains relatively unexplored. These monomers possess the following advantages: (a) they are liquid at room temperature and, therefore, can be applied without the need of solvent; (b) Although these polymers have low viscosity at room temperature, they can rapidly harden when crosslinked; (c) They possess a higher number of potentially reactive end groups per molecule compared to high molecular weight multi-armed polymers or linear polymers of similar molecular weight; (d) It is relatively easy to control the viscosity of the material by varying the molecular weight and/or crosslinking degree; and (e) They have low immunogenicity and toxicity. In this presentation I will discuss strategies for designing neat pre-polymers for medicine and biotechnology as well as new concepts in drug delivery and tissue reconstruction.

SMART MICELLAR NANOCARRIERS

Roey Amir, Assaf Harnoy, Ido Rosenbaum, Liat Frid, Marina Buzhor, Segal Merav, Yael Cohen
School of Chemistry, Tel Aviv University

Stimuli-responsive micelles that can disassemble and release their encapsulated cargo upon external stimuli have gained increasing attention due to their possible utilization as nanocarriers for drug delivery. Among the various types of stimuli, enzymes offer great potential for the activation of biomedical carriers due to their overexpression in various diseases. In this talk we will report a highly modular design for the simple and efficient synthesis of amphiphilic block copolymers based on a linear hydrophilic polyethyleneglycol (PEG) and an enzyme-responsive hydrophobic dendron. The PEG-Dendron hybrids were synthesized through divergent synthetic methodology using a combination of amidation and tiol-yne reactions. These amphiphilic hybrids were found to self-assemble in water into micellar nanocontainers that disassembled and released encapsulated molecular cargo upon enzymatic activation. The modularity of these PEG-dendron hybrids offers control over the disassembly rate of the formed micelles by simply tuning of the PEG length. Such smart amphiphilic hybrids could potentially be applied for the fabrication of nanocarriers with adjustable release rates for delivery applications.

STIMULI RESPONISIVE SYSTEMS IN DRUG AND GENE DELIVERY APPLICATIONS

Yosi Kost
Department of Chemical Engineering, Ben-Gurion University of the Negev, Israel

The basic approach that drug concentration-effect relationships are significantly invariant as a function of time in man has led to the development of constant rate drug delivery systems. Nevertheless, there are a number of clinical scenarios where such an approach may not be sufficient. Thus, in recent years several research groups have been developing stimuli responsive systems that could more closely resemble the normal physiological process. The stimuli responsive polymeric delivery systems can be classified as open or closed-loop systems. The open-loop devices apply external stmuli for on demand or targeted delivery such as: magnetic, ultrasonic, thermal, electric and chemical/biological substrate. In closed-loop devices the release rate is controlled by feedback information, without any external intervention. The self-regulated systems utilize several approaches as rate control mechanisms: pH-sensitive polymers, enzyme-substrate reactions, pH-sensitive drug solubility, competitive binding, antibody interactions, and metal concentration dependent hydrolysis. Open and closed loop systems studied in our lab would be preseneted.

ENHANCED ORAL BIOAVAILABILITY OF HYDROPHOBIC DRUGS

Abraham Domb, Abraham Domb, Amnon Hoffman, Moran Haimzada, Ira Cherniakov
School of Pharmacy, The Hebrew University of Jerusalem, Israel

Pro-nano Liposphere (PNL) formulation based on a solution of a hydrophobic drug in a mixture of surfactants, water miscible solvent and solid lipids that upon introduction to stomach fluids forms lipid nanoparticles, have been developed. The oral bioavailability of cyclosporin has been improved by this pro-nanodispersion liposphere formulation, allowing 25% oral bioavailability of cyclosporin in humans. The bioavailability of cyclosporine was enhanced in correlation with the reduction in the size of the formed nanoparticle. These liposphere formulations have been used to improve the oral bioavailability of drugs suffering from first pass metabolism such as CBD and THC. Nano-lipospheres enhanced CBD's oral bioavailability by increasing solubility and reducing Phase I metabolism. This novel approach, that enables such a co-localization, provides additional 2-fold increase in CBD's bioavailability as compared to CBD-PNL. This additional augmentation in the absorption can be most probably attributed to Phase II glucoronidation process inhibition at the enterocyte level by piperine in addition to the Phase I inhibition by PNL. Another formulation based on biodegradable nanoparticles for nasal spray administration have been used for delivering short peptides to the brain. The clinical development involved with these technologies will be discussed.

POLYMER NANOMICELLES AS A PLATFORM FOR ORAL DRUG DELIVERY IN CANCER

Ofra Benny
Institute for Drug Research, The Hebrew University, Israel

Oral administration is the preferable route of drug delivery, especially in chronic diseases. However, drugs that are classified as poorly soluble and permeable present a significant challenge. The gastrointestinal track, with its wide range of pH and enzymatic activity, is a substantial physiological barrier for drug availability.

With the emerging clinical use of drugs that target the tumor microenvironment in cancer, the need to develop formulations that are suitable for long-term chronic therapy became crucial. Unlike chemotherapy that is commonly used systemically at the maximum tolerated dose, maintenance therapies in cancer aim to sustain a minimally toxic but active drug level.

For the goal of developing a platform technology for oral drug delivery of drugs with poor bioavailabilty, we utilize a nanomedicinal-approach for drug delivery. A short co-polymer polylactic acid-polyethylene glycol (PEG-PLA) was used to successfully carry hydrophobic drugs in a compact self-assembled nanomicelle structure. These unique nanomicelles can be used for either conjugation or encapsulation of drugs, depending on the desired properties and application. As an example, we show a successful development of an oral formulation of a broad spectrum anti-angiogenic drug which originally presented very low bioavailability. In its nanomicelle form, the drug not only maintained its activity but also showed preferable tumor accumulation, reduced toxicity and improved stability.

In our current study, we reveal the molecular mechanism which mediates the intestinal absorption of PEG-PLA nanomicelles using caco-2 permeability assay and a high-resolution TEM imaging of mouse-intestinal specimen. Our findings broaden the possibilities of using nanomicelles for oral drug delivery and carry significant clinical relevance in the arena of chronic therapy in cancer and other diseases.

LOCALIZED STIMULATION OF DIFFERENTIATION IN A HUMAN EMBRYOID BODY MODEL USING MICROSPHERE-COUPLED FACTORS

Amir Fine, Rebecca Khalandovsky, **Shulamit Levenberg**
Biomedical Engineering, Technion, Israel

Embryonic stem (ES) cells have the ability to differentiate into all germ layers, with significant potential in both early embryonic development modeling and as a robust cell source for cell-replacement therapies and drug screening assays. While ES cells are commonly grown as embryoid bodies (EBs), the 3D organization and structure of EBs presents unique challenges that hinder regulation of the differentiation processes. As ES cell differentiation is strongly influenced by physical and chemical signals emanating from the local extracellular microenvironment, current EB differentiation techniques primarily focus on regulating spatial control of EB size, as well as culture and environmental conditions. In order to investigate the mechanisms that control ESC differentiation and to identify the cues that trigger germ layer differentiation, we developed a new means of controlling human ES cell differentiation in the three dimensional EB system. Fluorescent BMP4-coupled microspheres were incorporated into human EBs, enabling control of localized differentiation induction from within the EB. Expression of the early mesoderm marker Brachyury was then monitored and localized relative to the fluorescent microspheres. Our results demonstrate the potential of the new approach in directing differentiation of human ES cells in a three dimensional system and as a novel system for following differentiation at the single-cell level within the EB.

DO NOT FORGET THE FORGOTTEN DISEASES…AND PATIENTS

Sosnik Alejandro
Materials Science and Engineering, Technion, Israel

Contrary to cancer that affects people in both developed and developing countries almost indistinctly, most epidemic infectious diseases (e.g., tuberculosis, HIV/AIDS, malaria) show dramatically higher incidence in poor countries. This heterogeneous group of maladies has been coined poverty-related diseases (PRDs) and, in many cases, their gold-standard therapy remained unchanged over decades due to limited market profitability. This, regardless of the low efficacy rates and/or the use of administration regimens and routes that are patient incompliant. Also, morbidity and mortality in some patient sub-populations is often higher, demanding especially tailored treatment programs. For example, the High Activity Antiretroviral Therapy (HAART) has showed very good results to prevent the progress of the HIV infection to the active phase, AIDS. More recently, the efficacy of HAART to reduce transmission rates among high-risk individuals has been demonstrated, expanding the applications of the therapeutic cocktail. However, complicated administration schedules impact the patient lifestyle and favor treatment cessation. Due to the lack of approved drugs and appropriate pediatric formulations, the treatment of HIV-infected children is even more challenging. While technology interventions entail a promising strategy to overcome the most common biopharmaceutical drawbacks and improve the performance of approved and pipeline drugs, innovation in PRDs is scarce and none of the developments has reached the market yet. This presentation will discuss the challenges faced to innovate in PRDs from a scientific but also from an ethical perspective and some strategies that could contribute to make innovative medicines more translatable into clinics and affordable.

ADVANCED BIOMATERIALS FOR CARDIAC TISSUE ENGINEERING

Tal Dvir
Biotechnology, and Materials Science and Engineering, Tel Aviv University

The heart is a non-regenerating organ. Consequently, the loss of cardiac cells and formation of scar tissue after extensive myocardial infarction frequently leads to congestive heart failure. Given the scarcity of cardiac donors, a potential approach to treat the infarcted heart is to repopulate the 'dead zone' with cells capable of spontaneous contraction. Cellular therapy evolved to introduce cells into diseased areas and regain function. However, two main drawbacks of this approach are the lack of control of cell accumulation site after injection, and cell death before forming cell-cell or cell-matrix interactions. These shortfalls motivated the development of the tissue engineering concept, where 3-dimensional (3D) biomaterials serve as extracellular matrix-like scaffolds to the cells, enabling the cells to assemble into effective tissue substitutes, that may restore tissue or organ function. After transplantation the scaffolds either degrade or metabolize, eventually leaving a vital tissue instead of the defected tissue. In this talk I will describe cutting-edge technologies for engineering functional cardiac tissues, focusing on the design of new biomaterials mimicking the natural microenvironment of the heart, and the use of inorganic nanostructures and devices for actuating and monitoring tissue performances *in vitro* and *in vivo*.

LIPOSOMAL DELIVERY OF A LIPOPHILIC ANTI-CANCER PRODRUG: FROM BENCH TO BEDSIDE

Alberto Gabizon[1,2], Yogita Patil[2], Hilary Shmeeda[1], Yasmine Amitay[3], Patricia Ohana[3]
[1]*Oncology, Shaare Zedek MC, Israel*
[2]*Oncology, Hebrew University-School of Medicine, Israel*
[3]*Pharm. Inc., Lipomedix, Israel*

A strategy for liposomal drug delivery is to use a lipophilic prodrug with affinity for the liposomal lipid bilayer. A mitomycin-C lipid-based prodrug (MLP) is efficiently entrapped in pegylated liposomes (PL) and, under proper conditions, can be released and activated by thiolytic cleavage generating free mitomycin C (MMC). This formulation (PL-MLP or Promitil®) has reduced toxicity and an improved therapeutic index as compared to MMC in human and mouse tumor models. Pharmacokinetic studies in various species (mice, rats, pigs) revealed major (100-fold) differences in Cmax, AUC, plasma clearance and volume of distribution between MMC and PL-MLP in animal studies. PL-MLP has completed Phase I clinical testing. 27 cancer patients received 101 once-monthly infusions (median=3 cycles/patient; range=1-12). The highest dose level tested was 3.5 mg/kg (=1.03 mg/kg MMC-equivalents). The single dose MTD (3 mg/kg) of PL-MLP in MMC-equivalents is ~3-fold greater than for MMC. The pharmacokinetics is characteristically Stealth-like as with other pegylated liposomes. PL-MLP has an excellent safety profile at doses of the prodrug exceeding the maximal tolerated dose of equivalent doses of mitomycin C. Pharmacokinetic analysis indicates MLP has a slow, nearly mono-exponential clearance, with a long circulation half-life of ~1 day and a small volume of distribution similar to blood volume. The high plasma levels of the prodrug detected after infusion of PL-MLP indicate that the formulation is stable in the blood stream with minimal prodrug activation and release of MMC in circulation. Yet, its significant antitumor activity in various tumor models is proof of in vivo prodrug activation in tumors. Encouraging results of recent animal studies suggest that combining PL-MLP with other chemotherapeutic agents greatly improves anti-tumor efficacy without compromising safety. PL-MLP may represent an effective therapeutic tool in a broad spectrum of malignancies, including multi-drug resistant tumors.

EFFECTS OF SLOW RELEASE OF QUORUM SENSING INHIBITORS ON VIRULENCE IN PSEUDOMONAS AERUGINOSA

Michael Meijler, Hagit Forckosh, Antonia Delago
Department of Chemistry and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Israel

Quorum sensing (QS) enables unicellular organisms to coordinate their behavior and function in such a way that they can adapt to changing environments and compete, as well as coexist, with multicellular organisms. Recently we have developed tools to gain insight - through labeling and visualization - in the molecular mechanisms behind signal perception and regulation, in pathogens such as *P. aeruginosa* and *S. aureus*. We have fine-tuned this strategy, based on reactive inhibition of QS receptors, to successfully block QS in these pathogens as well as in *V. cholerae*. In addition, we have discovered QS inhibitors present in plant smoke, and these compounds appear to protect the plants effectively from infection by certain plant pathogens. An outstanding question in the field of QS inhibition is how exactly the slow release of QS inhibitors into growth medium or at the site of colonization affects bacterial virulence and ability to establish robust colonies. We address this question using basic model systems, evaluating the production of virulence factors such as pyocyanin and the formation of biofilms by *P. aeruginosa*.

NOVEL POMEGRANATE OIL NANO-EMULSIONS FOR THE PREVENTION AND TREATMENT OF NEURODEGENERATIVE DISEASES: THE CASE OF GENETIC CJD

Gabizon Ruth[1], Michal MIzrahi[1], Yael Friedman-Levy[1], Liraz Larush[2], Kati Frid[1], Orli Binyamin[1], Dvir Dori[1], Nina Feinstein[1], Haim Ovadia[1], Tamir Ben-Hur[1], Shlomo Magdassi[2]

[1]*Neurology, Hadassah University Hospital, Israel*

[2]*Chemistry, Hebrew University, Israel*

Background: Since disease progression in neurodegenerative diseases is associated with irreversible brain damage, prevention of disease onset and aggravation by safe compounds in at risk individuals should be the main target in treatment development. This is the case for carriers of mutations in the PrP protein, causing genetic Creutzfeldt-Jacob disease (gCJD).

Methods: To this effect and since sensitivity to oxidative stress plays a major role in prion and other neurodegenerative diseases, we treated our TgMHu2ME199K mice, which model for patients expressing the E200K PrP mutation in gCJD, with Pomegranate seed oil (PSO) in its natural form or in a novel emulsified formulation (Nano-PSO). PSO comprises large concentrations of a unique polyunsaturated fatty acid, punicic acid, considered as one of the strongest natural antioxidants. Young and asymptomatic mice, as well as sick TgMHu2ME199K mice were treated for months with PSO or Nano-PSO.

Results: We show here that administration of Nano-PSO significantly delayed disease onset in asymptomatic TgMHu2ME199K mice and postponed disease aggravation in already sick mice. Biochemical and pathological analysis revealed that while Nano-PSO did not affect PrP accumulation, it reduced lipid oxidation and neuronal loss and restored neurogenesis, indicating a strong neuroprotective effect.

Conclusions: Nano-PSO formulations maybe beneficial to subjects suffering from diverse neurodegenerative conditions, including prion diseases. They may be used as stand alone drugs or in combination with disease specific reagents.

RELEVANT PHYSICOCHEMICAL AND BIOPHYSICAL CHARACTERISTICS AS THE FOUNDATION OF THE DEVELOPMENT AND APPROVAL OF NANO-DRUGS

Yechezkel Barenholz

The lab of Membrane and Liposome Research, The Hebrew University Hadassah Medical School, Israel

There are many types of nano-particulate-based nano-drug formulations, including liposomes, polymeric and non-polymeric micelles, protein micelles, sub-micron- and nano-emulsions, dendrimers, nano-tubes (various kinds), and polymeric drug conjugates. Some of these can be pegylated in order to increase their circulation time, while their nano-size enable an EPR- based passive targeting, these nano-drugs can also include a desired ligand for active targeting to specific cells.

All nano-drugs are by definition super-molecular assemblies which complicates their development and their approval for clinical use. The experience gained during the last 25 years of nanotechnology and nano-drug development and extensive evaluation demonstrates clearly the obligatory need for applying an understanding of the cross-talk between physicochemical, nanotechnological, and biological principles. This is why the regulatory agencies such as FDA and EMA require an in-depth physicochemical characterization as a major part of the CMC, pharamaceutical development (including QbD) and of the IVIVC (www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/UCM199635.pdf). My presentation is aimed to share with the audience some of main tools used in order to deal with the physicochemical characterization that is so crucial for the successful development of nano-drugs. The major tools to be discussed include: size and morphology distribution analyses (which are not straightforward), X-ray diffraction (SAXS and WAXS), calorimetry (ITC and DSC), quantitative electron microscopy), and drug release from the formulation (equivalent to dissolution assay).

These will be demonstrated for two nanodrugs, one based on nano-liposomes and the second based on protein micelles.

*The Abstracts/Posters of Erez Koren et al., Tal Berman et al., Genia Levinton-Shamuilov et al., Lisa Silverman and Yechezkel Barenholz, Yaelle Bavli et al., Xiaohui Wei et al., Yealle Schilt et al., are directly relevant to this presentation.

#I acknowledge the contributions of:

Uri Raviv, Yaelle Schilt; Oren Regev, Sivan Peretz; Dganit Danino; Xiaohui Wei and Rivka Cohen; Aba Priev; Hadas Perlstein; Erez Koren;

DONOR-TWO-ACCEPTORS DYE DESIGN - A DISTINCT GATEWAY TO NIR FLUORESCENT PROBES

Doron Shabat
School of Chemistry, Tel Aviv University, Israel

Inspired by the quinone-methide elimination mechanism, we have shown how an intramolecular charge transfer can form unique quinone-methide type derivatives with a donor-two-acceptors molecular structure. This intramolecular charge transfer produces a fluorochrome with an extended π-conjugated system that could be used for the design of long-wavelength fluorogenic probes with a turn-ON option. One such probe was successfully used to image hydrogen peroxide *in vivo* in a mouse inflammation model. The donor-two-acceptors concept was translated to a library of new dyes with long-wavelength fluorescence emission. The ability of such dyes to emit NIR fluorescence through a turn-ON activation mechanism makes them promising candidate probes for *in vivo* imaging applications. This concept was recently applied to monitor real-time activation of a chemotherapeutic theranotic prodrug. We anticipate that our unique strategy will open a new door for further NIR fluorescence probe discovery.

CHARACTERIZATION OF CATHEPSIN ACTIVITY IN VULNERABLE ATHEROSCLEROTIC PLAQUE MACROPHAGES AND APPLICATIONS IN IMAGING AND THERAPY

Galia Blum[1], Ihab Abd-Elrahman[1], Karen Meir[2], Hisanori Kosuge[3], Yael Ben-Nun[1], Tommy Weiss Sadan[1], Chen Rubinstein[5], Yaacov Samet, Yaacov Samet[5], Mathew Bogyo[4], Mathew Bogyo, Michael McConnell[3]

[1]*Pharmacy, Hebrew University, Israel*

[2]*Pathology, Hadassah Medical Center, Israel*

[3]*Cardiovascular Medicine, Stanford University, USA*

[4]*Pathology, Stanford University, USA*

[5]*Departments of Vascular Surgery, Hadassah Medical Center, Israel*

Background: Atherosclerosis is a leading cause of mortality worldwide. Early detection and characterization of atherosclerotic plaques would greatly improve the diagnosis and clinical management of the disease. Current clinical imaging capabilities are limited to overall identification of plaque structure and stenosis; however, they poorly predict the risk of plaque rupture.

Objective: To improve current diagnostic tools for atherosclerosis and to test a cathepsin inhibitor as potential therapeutic.

Methods: We have applied novel fluorescent activity-based probes to assess cathepsin activity in human carotid plaques of progressive severity from patients with and without symptoms. We also evaluated the probes as reagents for non-invasive atherosclerosis imaging and preformed biochemical investigation of individual cathepsins in human plaque tissue. We then applied a potent cathepsin inhibitor to induce macrophage apoptosis.

Results: We first demonstrate that our technology accurately detects mouse atherosclerotic plaques non-invasively, identifying cathepsin activation within plaque macrophages. We then analyzed carotid plaques from patients undergoing endarterectomy that were pathologically graded, and show that our method detects up to 3-fold higher activity in unstable carotid plaques when compared to stable plaques. Furthermore, we demonstrate higher cathepsin activity in plaques from symptomatic patients. To better understand the plaque dynamics of cathepsin activity, we classified the cathepsin expression pattern and identified a unique up-regulation signature for cathepsin B and cathepsin S activities with progressive increase in plaque severity. Additionally, we demonstrate that M2 macrophages from unstable plaques express 1.5-fold higher cathepsin activity than M2 macrophages from stable plaques. Finally, we were able to induce selective macrophage cell death by treatment of intact plaques with our novel cathepsin inhibitor.

Conclusion: Taken together, our results present a powerful diagnostic tool for the characterization of high-risk human atherosclerotic plaques, elucidate a novel cathepsin activity pattern, and suggest a potential treatment for atherosclerosis.

IN VIVO TARGETING OF HYDROGEN PEROXIDE BY ACTIVATABLE CELL-PENETRATING PEPTIDES

Roy Weinstain[1], Elamprakash N. Savariar[1], Csilla N. Felsen[1], Roger Y. Tsien[1,2,3]
[1]*Pharmacology, University of California San Diego, USA*
[2]*Chemistry and Biochmistry, University of California San Diego, USA*
[3]*Howard Hughes Medical Institute, University of California San Diego, USA*

A growing body of evidence suggests that hydrogen peroxide (H_2O_2) plays an active role in the regulation of normal physiological processes. Nevertheless, its overabundance results in oxidative stress that can lead to extensive cellular damage. Indeed, high levels of H_2O_2 have been implicated in many pathological conditions including diabetes, cardiovascular and neurodegenerative diseases and cancer. Consequently, there is interest in deciphering the roles of H_2O_2 in normal and pathological conditions, as well as in its potential as a target in directed therapeutics delivery for oxidative stress related diseases.

To address these, a H_2O_2 targeting mechanism was developed based on activatable cell-penetrating peptides (H_2O_2-ACPP). Labeling of the H_2O_2-ACPP with donor and acceptor fluorophores enabled visualization of its reaction with H_2O_2 through ratiometric fluorescence emission due to loss of fluorescence resonance transfer (FRET). The H_2O_2-ACPP reacts selectively and in a concentration-dependent manner with H_2O_2 to release its CPP domain, whose adhesiveness and nondiffusibility preserve spatial resolution. Its low micromolar sensitivity (~ 5 μM) enabled detection and quantification of H_2O_2 secreted by activated HL-60 cells. Moreover, the H_2O_2-ACPP was sensitive enough to react with endogenous levels of H_2O_2 in an *in vivo* model of lung inflammation.

Developing ACPPs for H_2O_2 targeting will potentially enable its imaging by a variety of modalities, including fluorescence, magnetic resonance and radioactive techniques. Importantly, a similar targeting mechanism could be further used for directed delivery of therapeutics to local sites of oxidative stress related diseases.

THE APPLICATION OF CONVECTION-ENHANCED DRUG DELIVERY FOR THE TREATMENT OF BRAIN TUMORS – ADVANTAGES AND CHALLENGES

Yael Mardor
The Advanced Technology Center, Sheba Medical Center, Israel

Malignant primary brain tumors, especially the highly-malignant recurrent Glioblastoma multiforme, are fatal within months of diagnosis. Chemotherapy confers no significant survival advantage, in part due to the poor penetration of most chemotherapeutic drugs across the blood-brain barrier (BBB). Convection-enhanced drug delivery (CED) is a novel approach to deliver drugs directly into brain tumors that has shown great promise in advanced clinical studies. It is based on delivering a continuous infusion of drugs via intracranial catheters, enabling the convective distribution of high drug concentrations over large volumes of tissue while avoiding systemic toxicity.

Efficient CED formation depends on various physical/physiological parameters. Previous CED-based clinical trials showed a significant diversity in the extent of convection among patients and drugs. In addition, efficient CED is mostly obtained for small molecules (200 KDa).

Efficient CED of nanoparticles may enable the use of drugs and/or drug carriers that until now were considered inappropriate for convection treatment. Nanoparticles as drug carriers may enable real-time depiction of particle distribution in the tissue, provide slow drug release, enable the delivery of larger therapeutic agents, and allow targeted drug delivery.

The presentation will review the current status of CED for the treatment of brain tumors with emphasis on the challenges and advantages related to the delivery and monitoring of nano-carriers for controlled drug release.

PEPTIDE NUCLEIC ACIDS (PNA) AS ANTI-MALARIA AGENTS

Eylon Yavin
School of Pharmacy, The Hebrew University of Jerusalem, Israel

One of the major concerns in treating malaria by conventional small drug molecules is the rapid emergence of drug resistance. Specific silencing of essential genes by antisense oliogomers has been proposed as an alternative approach that may result in antimalarial activity which is not associated with drug resistance.

In addition, such an approach could be an important biological tool for studying many genes' function by reverse genetics. Here we present a novel methodology of using peptide nucleic acids (PNAs) as a useful tool for gene silencing in Plasmodium falciparum. PNAs, designed as specific antisense molecules, were conjugated to a cell penetrating peptide (CPP); namely, octa-D-lysine via the C-terminus, to allow facile delivery through cell membranes. PNAs added to *P. falciparum* cultures were found exclusively in infected erythrocytes and were eventually localized in nuclei of the parasites at all stages of intra erythrocytic development.

We show that these PNAs specifically down regulated both a stably expressed transgene as well as an endogenous essential gene, which significantly reduced parasites' viability. This study paves the way for a simple approach to silence a variety of *P. falciparum* genes as means of deciphering their function and potentially to develop highly specific and potent antimalarial agents.

ANIONIC POLYPLEXES FOR SIRNA DELIVERY

Smadar Cohen
Biotechnology Engineering, Ben-Gurion University, Israel

Sub-cellular targeting of siRNA can drastically improve its efficacy while minimizing side effects. Here, I will describe the development of anionic polyplexes formed between siRNA and hyaluronan-sulfate and mediated by calcium ion bridges. In addition to their potential biocompatibility compared to cationic carriers, the anionic siRNA polyplexes present several advantages; the simple preparation method under aqueous conditions ("green technology") enables mass production; and the use of hyaluronan-sulfate can enhance their targeting and uptake by certain cells carrying the HA receptor. Using this platform for delivering anti-EGFP siRNA, 85% silencing of EGFP was observed in EGFP-transfected CT26 mouse cell cultures. EGFP knockdown corresponded with the substantial cell uptake of these polyplexes and their accumulation and targeting to the cell cytoplasm. Similar levels of gene silencing of a more relevant therapeutic target (STAT3 transcription factor) were demonstrated in two human cancer cell lines: multiple myeloma (U266) and hepatocellular carcinoma (HepG2) as well as in primary cell cultures.

Gamma-MAGHEMITE-POLYMER HYBRID NANOCOMPOSITES FOR SIRNA/MICRORNA DELIVERY/GENE SILENCING APPLICATIONS: INNOVATIVE CHEMICAL STRATEGIES FOR TOXICITY CONTROL

Jean-Paul (Moshe) Lellouche[1], Liron Limor Israel[1], Stella Ostrovsky[1], Katya Buchman-Kapilov[1], Valeria Lia Yarmiyaev[1], Emmanuel Lellouche[2], Moshe Bechor[2], Shulamit Michaeli[2]

[1]*Dpt of Chemistry & BINA, Bar-Ilan University, Israel*

[2]*Faculty of Life Sciences & BINA, Bar-Ilan University, Israel*

Iron oxide nanoparticles (NPs) have been quite widely used in numerous biotechnology applications (magnetism-driven cell separation, magnetic field-guided drug/gene delivery, non-invasive tissue MRI, anti-cancer hyperthermia). Serious drawbacks dealing with NP fabrication, *i.e.,* both *detrimental NP aggregation* and *controlled NP surface functionalization versatility* including *intrinsic nanocarrier toxicity mitigation are* extremely challenging issues calling for innovative solutions.

Our recent work in the field led to the discovery of a novel method/concept for the (i) aggregation control of ultra-small hydrophilic super-paramagnetic maghemite (*gamma*-Fe_2O_3) NPs and for (ii) its successful use for NP functionalization toward siRNA/microRNA-mediated gene delivery/silencing applications. *This nanofabrication method does not make use of any surface-passivating organic species*. Indeed, the controlled high-power ultrasound-assisted metal Ce(III/IV) cation doping of the surface of 45/50 nm-sized (DLS) maghemite NPs strongly modified the NP surface charge to highly positive values (+41.0 - +53.0 mV range) of z□potential. Such a $Ce^{3/4+}$ cation-doping process enabled (i) an effective charge control of NP aggregation, (ii) the full NP water compatibility for biological applications, and finally (iii) the development of quite versatile surface engineering chemistries using the known rich $Ce^{3/4+}$ complex *coordination chemistry* for any biomolecule or organic species (polymer) binding.

This new NP "*inorganic*" stabilization and surface functionalization approach afforded optimized ultra-small core $Ce^{3/4+}$-doped *gamma*-Fe_2O_3 NPs leading to various hybrid 25kDa -PEI polymer-based decorated nanocomposite carriers (NCs) for siRNA/microRNA *in vitro/in vivo* delivery applications. In addition, effective chemical strategies for nanocarrier toxicity mitigation will be reported based on selected amine function chemical modifications (*N*-amidation, *N*-alkylation, selective amine group oxidation, etc…). Such NC surface modifications lead to quite effective *in vitro (screening step)/in-vivo* end-user applications dealing with safe siRNA/microRNA delivery.

CD44-TARGETED SYSTEMS FOR INTRACELLULAR DELIVERY OF CHEMOTHERAPEUTIC DRUGS AND SMALL INTERFIRING RNA (siRNA)

Ayelet David
Department of Pharmacology, The Faculty of Health Sciences, Ben-Gurion University of the Negev

CD44 is a transmembrane receptor expressed by many cell types, including leukocytes, fibroblasts, epithelial cells, keratinocytes and some endothelial cells. It has a variety of significant biological roles including maintaining tissue structure via cell–cell and cell–matrix adhesion, and mediating cell migration during morphogenesis, angiogenesis, tumor invasion and metastasis. Changes in CD44 expression are associated with a wide variety of tumors and the metastatic spread of cancer. In recent years, hyaluronic acid (HA) has emerged as a promising candidate for intracellular delivery of various therapeutic and imaging agents because its ability to specifically bind to various cancer cells that overexpress CD44 receptor. In addition to *HA*, *CD44 binds* fibrinogen, fibronectin, collagen, laminin, fibroblast growth factor-2 and other heparin-*binding* growth factors. The laminin derive peptide A5G27 (KLVSYNGIIFFLR) bind CD44 via it's glycosaminoglycan (GAG's) side chain, and was found to inhibit tumor cell migration, invasion, and angiogenesis in a dominant-negative manner. Thus, when attached to polymeric carrier A5G27 can be utilized both as therapeutic agent as well as a targeting peptide in order to navigate polymer-bound drugs and polyion complexes directly to the areas of malignancy, and inhibit tumor progression. In this lecture I will describe the design HPMA copolymer-Paclitaxel (PTX) conjugates (P-(A5G27)-PTX) and polyethylene glycol-block-polyethylene imine /siRNA complexes (A5G27F-PEG--PEI/siRNA) copolymer for targeted drug delivery to tumors that overexpress CD44. Using the *B16-F10* melanoma *lung metastasis* model we demonstrated that P-(A5G27)-PTX can increase the survival of tumor-bearing mice when compared to non-targeted control copolymer (P-(GG-OH)-PTX), indicating the advantage of the conjugation of A5G27 and PTX on the same polymeric backbone in improving antitumor efficacy. A5G27F-PEG-b-PEI block copolymer can selectively deliver siRNA into CD44-overexpressing cells for effective gene silencing, and its *gene silencing* potency is currently being *tested in vivo.*

NANO DELIVERY SYSTEMS OF HYDROPHILIC BIOMACROMOLECULES FOR IMPROVED THERAPY

Simon Benita, Orit Amsallem, Taher Nassar, Natalia Naraykin, Ouri Schwob, Eylon Yavin, Philip Lazarovici
Pharmacy, Hebrew University of Jerusalem, Israel

Hydrophilic biomacromolecules (proteins, peptides, oligonucleotides or siRNAs) usually exhibit poor membrane permeability and high sensitivity. They typically require the use of nanocarriers for efficient intracellular delivery. However, to date, the number of clinically relevant nano delivery systems used for such a purpose, are scarce. In this study, double nanoencapsulation was used to protect and control the release of siRNA, insulin and other peptides. The macromolecules were first incorporated into primary nanoparticles (NPs 70-100 nm) which were further encapsulated in sub-micron capsules (*i.e.* nanocapsules 1μm), using a novel technique of nano spray-drying, performed at low temperatures (60° C). This new method, developed in our lab, produces the final double nanocarriers in the form of dry powder (as confirmed by DSC measurements) whilst simultaneously preserving the loaded macromolecules (based on HPLC and gel electrophoresis assays).

The primary and secondary coatings of the nanoparticles consist of biodegradable polymers; HSA (human serum albumin) and PLGA (Poly D,L-lactic-co-glycolic acid) respectively, both approved for parenteral administration in humans. SEM, Cryo-SEM and laser scattering technologies were used for morphology, encapsulation, and particle size distribution evaluations. Adequate encapsulation of siRNA was achieved, and preliminary *in-vitro* kinetic experiments showed siRNA controlled release. Uptake of the primary NPs into A-431 human epithelial squamous carcinoma cells was observed after 4h, using confocal microscopy. Other macromolecules (peptides and antisense oligonucleotides) have been successfully double nanoencapsulated and preliminary animal results are encouraging. For example, streptozotocin diabetic SD rats exhibiting fasting glycemia above 250 mg/Dl, when injected with double nanoencapsulated insulin, elicited a marked prolonged decrease in blood glucose over 24 h in fasting conditions whereas in non-fasting conditions, the effect was shorter but still significant. If successful, this original nano-delivery system, will provide clinicians with an arsenal for controlled delivery of sensitive, potent, and short-acting biological drugs, for treating severe chronic diseases.

RNAi-BASED THERAPY FOR SOLID TUMORS - RESULTS FROM PHASE I/IIB IN PANCREATIC CANCER

Shemi Amotz
Silenseed LTD, Silenseed LTD, Israel

RNAi-based therapy presents a huge promise for many therapeutic areas. However, effective delivery of the RNAi-based drugs to the target tissue still remained a major challenge. Local and prolonged delivery of siRNA is a viable solution for many indications, specifically for solid tumors. Pancreatic cancer (PC) is an aggressive disease that leads to a high mortality rate. Over 90% of patients carry a mutation in K-Ras to which the tumor is addicted to. We developed an implantable system for controlled regional drug delivery, by designing a miniature biodegradable polymeric matrix that encompasses an anti-$KRAS^{G12D}$ siRNA drug, named *siG12D LODER*™. The LODER™ prevents siRNA degradation in-vivo along months, and releases the drug regionally within a pancreatic tumor during four months. Treatment of pancreatic cells with *siG12D LODER*™ resulted in a significant inhibition of KRAS mRNA and reduction in its protein levels. Decrease of KRAS inhibited cell proliferation and reduced EMT inducing protein levels. *In vivo,* in mice implanted with *siG12D LODER*™ the growth of human PC cell lines was retarded, the survival was significantly improved, and development of new metastasis was halted. Evidences from histology analysis showed drug distribution throughout the tumor in a typical rate of one mm per day. We completed a phase I clinical study with the *siG12D LODER*™, which was implanted into patients with locally advanced PC by using a standard endoscopic ultrasound device. The results of the clinical study show a high safety profile. Moreover, patients had an extending overall survival and a retardation of tumor progression. Multinational controlled Phase II clinical trial (NCT01676259) is now in progress. Next generations LODERs in which we have incorporated novel nano-technologies are in development, tailored to the specific requirements of tumors in cancers such as prostate and brain.

POSTERS SELECTED FOR SHORT PRESENTATIONS

TARGETED GOLD NANOPARTICLES IN THE TREATMENT OF PSORIASIS

Alona Goldstein, Yoram Soroka, Marina Frusic-Zlotkin, Ron Kohen
Institute for drug research, School of Pharmacy, The Hebrew University of Jerusalem, Israel

Psoriasis is a distressing, chronic skin disease which affects about 2 % of the world population and characterized by hyperproliferation of epidermal cells and a strong inflammatory process. Macrophages at their activated state are involved in stimulating the disease in the psoriatic lesions. Targeting those cells may be beneficial for the immediate relief of the acute stormy inflammatory process. Our aim is to develop a novel topical treatment for psoriasis by gold nanoparticles based on their abilities to undergo surface plasmons excitation under illumination at their resonance wavelength. This may lead to selective cell destruction and/or to the activation, directly and indirectly, of the Nrf2-Keap1 pathway leading to the transformation of the activated macrophages to their resting state. The suggested novel approach has the advantages of being a local treatment, non toxic, specific and could lead to an immediate relief.

Our findings demonstrate the ability of 5nm gold nanoparticles to penetrate the epidermal barrier and to reach the dermis. Macrophages in cell culture revealed an impressive uptake of gold nanoparticles. Selective apoptosis of macrophages was achieved by the combination of cell incubation with gold nanoparticles and local laser irradiation at 560nm wavelength. At lower laser intensity, apoptosis of the cells did not occur, but the generation of reactive oxygen species was observed within the cells. In addition, gold nanoparticles, even without laser irradiation, induced ROS generation. This suggests that 5nm gold nanoparticles by themselves already apply minor stress to the cells. Endogenous ROS generation may activate the Nrf2-Keap1 pathway to produce defense phase II enzyme that will reduce the inflammation and shift the activated macrophages to their resting state. Our aim is to further study the interaction between gold nanoparticles and macrophages, as well as their interaction with the skin, with and without plasmonic excitation by an external irradiation.

TARGETED THERAPY OF METASTATIC CANCER USING CELL DERIVED NANO-GHOSTS

Natali Malkah, **Stasia Krishtul**, Naama Toledano Furman, Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel

One major challenge in cancer therapy concerns the formation of metastasis, the spread of cancer cells from the initial tumor to other parts of the body. Despite the improvement in cancer treatment, many drugs fail to reach sites of metastases.

We propose to utilize Nano-Ghosts (NGs), our novel drug delivery system, to selectively target metastasis while delivering therapeutic drugs. NGs are vesicles produced from the plasma membrane of human mesenchymal stem cells (hMSCs), known for their natural targeting of multiple cancers and hypo-immunogenicity. As a model, we chose to target metastatic non-small cell lung carcinoma (NSCLC), the most common lung malignancy, which holds the highest mortality rate of all cancers. Two platinum based anticancer drugs were evaluated– cisplatin and carboplatin, the first being the drug of choice for treating NSCLC. Platinum based anticancer drugs are widely used in the clinic, treating a variety of tumors, however their clinical use has been impeded by their sever cytotoxicity, leading to agonizing side effects. Nanocarrier-based delivery is one approach trying to address this issue, in order to improve current platinum chemotherapy.

We were able to design a NGs system encapsulating these two drugs, while using two drug encapsulation methods: extrusion and electroporation. TEM imaging assured the formation of the Pt-NG and dynamic light scattering analysis confirmed that the NGs average diameter size was 260 nm and average zeta potential was -17 mV. Drug: lipid ratio and encapsulation efficacy rates of cisplatin were determined using ICP, and found to be highest using electroporation (0.15 µg/µg and 6.6%, respectively). Cisplatin showed higher stability when dissolved in saline as compared to DMSO. Interestingly, the results show that incorporation of platinum drugs in NGs, improved their therapeutic index toward NSCLC cell line A549 compared to the free drug (30% differences in cell viability).

DEVELOPMENT OF MODIFIED STARCH BASED COMPLEXES FOR PI3P DELIVERY IN ORDER TO OVERCOME INSULIN RESISTANCE

Nitzan Marelly[1], Tali Vodonos[2], Riki Goldbart[1], Tamar Traitel[1], Asaf Rudich[2], Joseph Kost[1]

[1]*Department of Chemical Engineering, Ben Gurion University of the Negev, Israel*
[2]*Department of Clinical Biochemistry, Ben Gurion University of the Negev, Israel*

Autophagy, an intra-cellular process in eukaryotic cells, allows for the digestion and recycling of cytoplasmic contents. This is achieved through the formation of double-membrane vesicles (autophagosomes) that undergo degradation through fusion with lysosomes. It has been suggested that dysregulation in autophagy contributes to different common human disorders. For example, a decrease in autophagy flux is correlated with insulin resistance of hepatocytes in obesity. This may be solved by up-regulating autophagy flux[1].

It was shown that Phosphatidylinositol 3 phosphate (PI3P), mediates autophagosome biogenesis through membrane deformation and elongation, and therefore acts as an activator of autophagy[2]. A major obstacle in delivering exogenous PI3P into cells is overcoming its negative charge (derived from the phosphate groups on the inositol ring).

Starch is a natural polysaccharide that is considered advantageous for drug delivery due to its biodegradability, biocompatibility, low immunogenicity and minimal cytotoxicity[3]. In this research, modified cationic starch is used as PI3P carrier. The positively charged quaternized ammonium groups on the modified starch and the negatively charged PI3P interact electrostatically, allowing for complexation.

Quaternized starch and PI3P complexes (Q-Starch/PI3P) radius, geometry and surface charge were evaluated by Dynamic Light Scattering, Zeta Potential and Atomic Force Microscopy. Molar ratio of Q-Starch nitrogen groups and PI3P phosphate groups (N/P ratio) was varied in the range of 1 to 3. Results show that increasing N/P ratio has no significant affect on complex size, with an average mode radius of ~35nm. In contrast, a mild increase in average surface charge appears (8.94-13.46mV) with increasing N/P ratio. Similar values have been previously shown to allow for cellular uptake. Indeed, preliminary results of *in-vitro* experiments show intra-cellular uptake of Q-starch/PI3P complexes. This was evaluated by confocal microscopy and ImageStream X of HEK-293 cells treated with Q-Starch/PI3P complexes.

ANTICANCER POLYMERIC NANOMEDICINE BEARING SYNERGISTIC DRUG COMBINATION

Hemda Baabur-Cohen, Ela Markovsky, Ronit Satchi-Fainaro
Physiology and Pharmacology, Tel Aviv university, Israel

Multivalent polymeric systems are an ideal platform for combination therapy, where the therapeutics are given simultaneously in one injection and share the same pharmacokinetic profile (*1*). We have found that the combination of the microtubule-interfering agent, paclitaxel (PTX) and the anthracycline antibiotic, doxorubicin (DOX) displays synergistic cytotoxic effects on cancer cells, such as the human breast cancer cell line, MDA-MB-231, and the human ovarian cancer cell line, ES-2.

Drugs conjugation with a nano-sized polymer enables preferred tumor accumulation by passive targeting, making use of the enhanced permeability and retention (EPR) effect (*2*). Polyglutamic acid (PGA) is a water-soluble multivalent polymer, non-immunogenic, non-toxic at the concentration required for its anticancer activity and is biodegradable by cathepsin B, an enzyme that is highly expressed in most tumor tissues.

We developed a new strategy for combination therapy for the treatment of breast and ovarian cancers. PGA-PTX-DOX nano-conjugate was synthesized and evaluated *in vitro* and *in vivo*. PGA-PTX-DOX inhibited both the proliferation and migration of MDA-MB-231 and ES-2 cells. Furthermore, our novel conjugate inhibited the growth of mammary tumors, inoculated orthotropically in nu/nu mice by 92%, compared to 64% inhibition achieved by the mixture of the two conjugates (*3*). Our results with PGA-PTX-DOX nano-conjugate present its potential use as a novel combination therapy for breast and ovarian cancers.

REFERENCES

1. E. Markovsky *et al.*,*J Control Release* **161**, 446 (Jul 20, 2012).

2. H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *J Control Release* **65**, 271 (Mar 1, 2000).

3. E. Markovsky, H. Baabur-Cohen, R. Satchi-Fainaro, *J Control Release* **187**, 145 (May 24, 2014).

*Equal contribution

RECOMBINANT IMMUNOTOXINS FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES

Nurit Becker, Itai Benhar
Biotechnology, Tel Aviv University

Antibody-mediated therapy of cancer has been more successful for hematological malignancies than for solid tumors. Due to tumor penetration limitations, small antibody fragments have an advantage over full IgG in those cases. Still, antibodies of the IgG format remained advantageous in other essential parameters such as longer circulation half-life, stability and manufacturability. While monoclonal antibodies can bind selectively to tumor cells, binding does not often lead to cytotoxicity unless a cytotoxic agent is attached to the antibody. Such immunotoxins are made by attaching a cytotoxic moiety to the antibody and the cell killing efficacy is significantly improved.

This study focuses on the comparison between the performance of full-length IgG antibodies and the small recombinant antibody fragment: disulfide – stabilized Fv (dsFv) as immunotoxins in hematological cancer therapy. Furthermore, we fuse the fluorescent protein m-cherry to the immunotoxins and created theranostic molecules, as they combine both: therapy and diagnostics. During this study we used the "Inclonals" method that was recently developed in our lab. This system enables the production of full-length IgGs in *E. coli*. The production of the IgG molecules in non-mammalian host provides the ability to express a cytotoxic moiety fused to it as a single polypeptide.

All of the immunotoxins and theranostic molecules were analysed for antigen and cell binding and for cell killing *In vitro*. The results show that *in vitro*, the smaller the molecule is – the more efficient it is. The pharmacokinetics of the smallest immunotoxin (dsFv-PE) and the biggest one (IgG(di)PE(di)mCherry) were tested *in vivo* and, as expected, the mouse serum $t_{1/2}$ of the IgG-based immunotoxin was 7.33 times than the $t_{1/2}$ of the dsFv-based immunotoxin. In order to compare its' therapeutic effect, the theranostic IgG and dsFv based molecules are now being analyzed *in vivo* in a human xenografts model in nude Mice.

LC100, A NOVEL PEGYLATED LIPOSOMAL DOXORUBICIN NANO-DRUG SUPERIOR TO DOXIL®: DESIGN, IN VITRO AND IN-VIVO CHARACTERIZATION

Tal Berman[1], Xiaohui Wei[2], Yaelle Schilt[3], Uri Raviv[3], Einat Nativ-Roth[4], Doron Friedman[5], Yechezkel Barenholz[1,5], Ilan Winkler[5]

[1]*Laboratory of Liposome and Membrane Research, IMRIC, Hebrew University-Hadassah Medical School, Israel*

[2]*School of Pharmacy, Shanghai Jiao Tong University, China*

[3]*Institute of Chemistry, The Hebrew University of Jerusalem, Israel*

[4]*Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Israel*

[5]*LipoCure, ltd, Israel*

Doxil® (pegylated liposomal doxorubicin) is the first FDA-approved (1995) nano-liposomal drug and is in extensive clinical use in cancer patients. Doxil's success is based on three principles: (i) prolonged drug circulation time and RES avoidance due to the PEG layer; (ii) high and stable remote loading of doxorubicin (by a transmembrane $[ammonium]_2$-sulfate gradient) that allows doxorubicin release at the tumor; (iii) lipid bilayer in a "liquid ordered" phase. The EPR effect "passively targets" Doxil to tumors. Doxil has a characteristic long and stable intraliposomal crystal, which imposes on the nano-liposome a transformation from a sphere to an ellipsoid, "coffee bean" shape.

Overcoming Doxil's adverse side effect of palmar-plantar erythrodysesthesia (PPE) and improving drug release at the tumor are expected to result in an improved therapeutic index. Our working hypothesis is that both can be improved by changing the physical state of the doxorubicin inside the nano-liposomal aqueous phase.

This was achieved by replacing the bivalent sulfate with the monovalent methanesulfonate, producing LC100: high and stable remote loading of doxorubicin by a transmembrane ammonium-methanesulfonate gradient, resulting in 90% encapsulation efficiency and chemical stability, both similar to Doxil. LC100 liposomes have a spherical shape unlike the ellipsoid Doxil.

From LC100, doxorubicin release at 37°C in the presence of buffer containing ammonium at pH 6.8 (imitating tumor microenvironment) and at pH 7.4 is much faster than from Doxil. In human plasma, release is negligible and resembles that of Doxil.

Methanesulfonic acid is FDA-approved for injection, as an excipient.

A study in a mice tumor model shows improved efficacy and a study in rats demonstrated statistically significant reduction in PPE, as well as a higher MTD. These advantages of LC100 over Doxil, demonstrate the feasibility of achieving a superior therapeutic index by controlling the intra-liposomal physical state of doxorubicin.

POSTER #6

TARGETING NANO-GHOSTS, CELL-DERIVED DRUG-CARRIERS, FOR THE TREATMENT OF INFLAMED TISSUES

Nitzan Letko, Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel

Targeted drug delivery systems have been the focus of many studies in recent years in hope to overcome obstacles associated with conventional drugs. After significant improvement in diagnostic and treatment of solid tumors, the question remains whether these systems can be translated to treat inflamed tissues.

As a model, we chose to target the inflamed myocardium, caused by myocardial infarction (MI), commonly known as a 'heart attack', which affects millions of people in the western world. MI is followed by cardiomyocytes death, extracellular matrix remodeling and acute inflammation in heart tissue, which then leads to the formation of a scar tissue and often results in congestive heart failure.

Human mesenchymal stem cells (hMSCs) are known for their unique properties. They home to inflamed tissues and sites of injury, and act as immunosuppressants. We produce nano-vesicles from the plasma membrane of hMSCs, termed Nano-Ghosts (NGs), and rely on the cells' natural abilities.

In the present work, we aim to target drug-loaded NGs to inflamed tissues, reduce inflammation and improve healing. NGs were prepared from hMSCs, resulting in spherical vesicles, with an average size of 200nm and a zeta potential of -16mV. NGs were shown to fully or partly preserve MSCs' surface markers, thus enabling NGs interactions with target cells. *In vitro* studies were conducted to investigate the interactions between NGs and various cells in the inflamed tissue, and an *in vivo* assay was performed to assess the targeting ability and accumulation of NGs in the injured myocardium, in a rat ischemia-reperfusion model. Indeed, NGs were detected in the inflamed heart tissue.

DESIGN, SYNTHESIS AND UTILLIZATION OF INTERNALLY FUNCTIONALIZED PEG-DENDRON HYBRIDS AS NOVEL DRUG DELIVERY PLATFORMS

Liat Frid[1], Dina Polyak[2], Merav Segal[1], Ronit Satchi-Fainaro[2], Roey Amir[1]
[1]*Exact Sciences, Tel Aviv university, Israel*
[2]*Physiology and Pharmacology, Tel Aviv university, Israel*

Despite the tremendous advances in the field of therapeutic delivery reported in last years, the demand for a more accessible, modular and highly efficient delivery systems is a continuing theme. The development of nanocarriers for the co-delivery of drug–nucleic acid combinations is considered the state of art in this area, due to its potential to achieve synergetic effects. Up to date, several dendritic delivery systems based on co-complexation of nucleic acids and drug molecules have been reported and tested for co-delivery. However, these delivery systems may lack the ability to release their molecular cargo in a particular sequence and time lags, which are often needed in order to achieve the desired therapeutic effects. To address these challenges, we designed and synthesized a novel PEG-dendritic hybrid delivery system that allows both the covalent binding of drugs and the complexation of nucleic acids through cationic surface groups. The high modularity of the presented system, allowed us to carry a preliminary evaluation of the structure-activity relation for several molecular architectures and to demonstrate their potential application as advanced delivery systems.

RECIPROCAL DORMANCY-PROMOTING NANOMEDICINE ALTERING EGFR AND TSP-1 FOR THE MANAGEMENT OF GLIOBLASTOMA

Shiran Ferber[1], Dikla Ben-Shushan[1], Galia Tiram[1], Orit Amsalem[2], Eylon Yavin[2], Nava Almog[3], Jack Henkin[4], Marcelo Calderon[5], Rainer Haag[5], Ronit Satchi-Fainaro[1]

[1]*Physiology and Pharmacology, Tel Aviv university, Israel*

[2]*School of Pharmacy Institute for Drug Research, The Hebrew University of Jerusalem, Israel*

[3]*Center of Cancer Systems Biology, Tufts University, USA*

[4]*Cemistry of Life Processes Institute, Northwestern University, USA*

[5]*Institut fur Chemie und Biochemie, Freie Universitat Berlin, Germany*

Glioblastoma (GBM) is one of the most aggressive human cancers. The current treatment involves surgical resection followed by radio- and chemo- therapies, resulting in median survival of 14 months. Due to GBM's invasive nature, recurrence occurs in 95% of the cases. Tumor progression is dependent on recruitment of blood vessels, as well as an established interaction with the surrounding microenvironment. Failure of a microscopic tumor to complete these stages may lead to delayed clinical manifestation and a non-progressing disease (*i.e.,* tumor dormancy). Residual tumor cells constitute fundamental clinical manifestation of tumor dormancy. Therefore, novel agents targeting relevant pathways are needed. We have previously generated two GBM dormancy models in mice. While the dormant avascular and fast-growing angiogenic tumor-forming cells share a similar growth rate *in-vitro*, we found profound differences in tumor growth patterns *in-vivo*. Two of the major dissimilarities were thrombospondin-1 (TSP-1), a key angiogenesis inhibitor, and epidermal growth factor receptor (EGFR), a modulator of tumorigenicity. The dormant tumor-generating cells express higher levels of TSP-1 and lower levels of EGFR compared to the fast-growing tumor-generating cells. Thus, they are considered attractive potential targets for therapy. Here, we induced upregulation of TSP-1, using a peptidomimetic (TSP-1-PM), and downregulation of EGFR, using a dendritic polymeric nanocarrier entrapping siRNA (PG-NH_2-siEGFR). This combination therapy's ability to reverse fast-growing angiogenic phenotype of the tumor to a dormant avascular phenotype was evaluated. Mice bearing fast-growing tumors received TSP-1-PM (50mg/kg daily) and PG-NH_2-siEGFR (2mg/kg bi-weekly). The treatment exhibited anti-angiogenic and anti-tumorigenic activities. It remarkably decreased tumor volume by 99.5% compared to control 25 days post treatment initiation to 1mm^3. Immunohistochemistry analysis revealed reduced vasculature, increased αSMA and decreased VEGF expression in treated tumors. We concluded that the combination of TSP-1-PM with EGFR-siRNA represents a promising treatment for advanced GBM promoting a dormant phenotype. *i.e.* stable asymptomatic disease.

THERANOSTIC BARCODED NANOPARTICLES

Zvi Yaari, Dana da Silva, Henn Kopal, Janna Shainsky Roitman, Avi Schroeder
Chemical Engineering, Technion, Israel

Selecting the proper drug, that addresses each patient's unique disease presentation, is the primary goal of personalized medicine. We describe here a nanoparticle-based barcoded system for predicting the therapeutic potency of drugs against cancerous lesions. The screen is performed inside the patient's body using extremely low drug doses, and grants insights regarding drug potency with single-cell sensitivity. This approach was tested on BALB/c mice bearing metastatic breast cancer tumors (4T1).

The diagnostic system is based on 100-nm liposomes loaded with a drug and a corresponding unique DNA barcode.

Once a tumor is detected, a cocktail of DNA-barcoded nanoparticles, each containing a different drug, is injected intravenously. The particles accumulate in the various cells that compose the tumor microenvironment, utilizing the enhanced permeability and retention (EPR) effect. Two days later, enabling each of the drugs to take action, a biopsy is taken from the tumor and the tissue is homogenized, to form a single-cell suspension. The cells are sorted by FACS according to cell type and to their live/dead viability state (potency screen). Then, the DNA barcodes are extracted from the cells and expanded using RT-PCR. The cell viability data is correlated with the type of drug/s found inside each of the cells, thereby identifying which drug or drug combination is optimal for treating the lesion.

Based on the screen, a treatment protocol was selected, generating a successful outcome in vivo. Interestingly, we found that barcodes can be detected even inside a single cell.

This approach, in which nanoparticles act as theranostic gauges for examining the therapeutic potency of a drug or drug combination may prove effective for personalizing medicine.

INTERACTIONS OF NANO-GHOSTS DERIVED FROM MESENCHYMAL STEM CELL WITH THE TUMOR NICHE

Yael Lupu-Haber, Naama Toledano-Furman, Limor Kaneti, Nitzan Letko, Tomer Bronshtein, Marcelle Machluf
Biotechnology and Food Engineering, Technion, Israel

The ultimate goal in cancer drug-delivery is producing a selective targeting system for cancer and its microenvironment. Targeted drug-delivery systems are designed to target the tumor, reduce side-effects and improve efficacy. These targeted systems are usually engineered by conjugating them with moieties that improve their selectivity.

In our lab we developed a targeted delivery platform based on unique cell derived nano-vesicles termed nano-ghosts (NGs) produced from whole membranes of mesenchymal stem cells (MSCs). We hypothesized that the NGs may preserve the targeting mechanism of the MSCs from which they are produced, as they benefit their surface markers. In-vivo, a single systemic administration of NGs originated from human or rat MSCs and loaded with a model therapeutic protein (sTRAIL) demonstrated unprecedented efficacy and achieved near complete prostate tumor inhibition.

In this work we aim to characterize the proteome of MSCs derived NGs and investigate the mechanism of interactions between the NGs and cells of the tumor niche (cancer, endothelial, and immune cells). Exploring the NGs proteome by flow-cytometry and mass-spectrometry reveals that MSCs surface markers are completely or partly retained. Furthermore, treating the MSCs with cytokines or cancer conditioned media prior to NGs production results in a similar effect on the expression profile of MSCs surface proteins and in an increased cellular uptake. Uptake of the NGs by endothelial cells, macrophages, and T-cells was also affected by stimulation of the MSCs. Cancer cells associate with the NGs through cellular uptake (endocytosis pathways) and by cellular binding (adsorption, lipid exchange, fusion). These interactions enable targeting of anti-cancer therapeutics to different compartments of the cancer cell. Indeed, viability assays show a higher cytotoxic effect of loaded anti-cancer therapeutics than the free therapeutics. Finally, using integrin antagonists results in inhibition of cellular uptake, suggesting integrins mediate part of the NG-cell interactions.

IMAGING OF VASCULAR-INJURED TISSUE BY LIPOSOMAL QUANTUM-DOTS

Gil Aizik[1], Nir Waiskopf[2], Nickolay Koroukhov[1], Uri Banin[2], Gershon Golomb[1]
[1]*School of Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Israel*
[2]*Institute of Chemistry, The Hebrew University of Jerusalem, Israel*

Several cardiovascular disorders, as other pathologies, are characterized by an inflammatory response, increased blood supply and massive monocytes infiltration to the injured tissue. This massive ingress to the inflamed tissue can be exploited to deliver therapeutic or diagnostic agents with high selectivity to the diseased tissue. This work examined a novel liposomal Quantum-Dots (LipQD) formulation to effectively accumulate, after been phagocytized by circulating monocytes, at the inflamed region. Negatively charged QDs, fluorescent nanocrystals with a composition of CdSe/CdZnS core/shell and glutathione capping, were encapsulated in positively charged liposomes. The uptake, stability and cytotoxicity were examined in murine monocyte/macrophage cell line, and in a rat model of vascular injury. High fluorescent intensities were observed, for at least 24 hrs, after local incubation of the carotid artery lumen of Sabra male rats with LipQD after arterial injury. In contrast, accumulation of QDs following incubation with free QDs suspension was not detected, at all time points, in the injured artery. In a preliminary study, a high fluorescent signal was seen in the injured artery of restenotic rats after intravenous administration of LipQD. The novel LipQD formulation offers a promising and attractive approach for inflammation imaging by exploiting the innate-immunity cells.

POSTER #12

A NOVEL LIPOSOMAL BUPIVACAINE FORMULATION TO PRODUCE ULTRALONG-ACTING ANALGESIA

Ilan Winkler[1], Nofar Keren[1], Katia Ioffe[1], Genia Levinton Shamuilov[1], Yaelle Bavli[2], Dima Shamrakov[1], Doron Friedman[1,2], Chezy Barenholz[2]

[1]*LipoCure Ltd, Jerusalem Biotechnology Park, Hadassah Ein Kerem, Israel*

[2]*Laboratory of Liposome and Membrane Research, IMRIC, Hebrew University-Hadassah Medical School, Israel*

Effective postsurgical pain management is a clinical imperative for every patient undergoing surgery. Infiltration of local anesthetics into the surgery site during closure provides temporary analgesia and is one aspect of the multimodal approach to postsurgical analgesia recommended in current guidelines. However, the duration of action of local "as is" anesthetics are limited. Patients may experience breakthrough pain before they are able to take or tolerate oral analgesics, thus necessitating the use of strong parenteral analgesics (frequently opioids) in the immediate postsurgical period. A higher total opioid dose increases the risk of experiencing an opioid-related adverse event, including life threatening apnea.

Therefore, an Ultra Long-Acting Local Anesthetic (ULALA) that facilitates prolonged peripheral nerve block and provides safe, efficacious analgesia with a single injection is desired (Mercado et-al, 2011).

LC400 is a two-stage delivery system in which Large Multi Vesicular Vesicles (LMVV) containing a large bupivacaine reservoir is embedded in a hydrogel. This drug delivery system was developed to provide two to three days of effective pain relief to patients suffering from severe trauma-induced pain (Cohen et-al, 2012, 2013).

Time-dependent bupivacaine concentration following LC400 local administration demonstrates a slower decrease of bupivacaine both at the injection site and in plasma, compared to that of un-encapsulated free drug.

LC400 has superior systemic toxicology and efficacy profiles, in comparison to free bupivacaine, with a minimal risk of adverse effects. This is achieved using a lower peak blood concentration, which allows a 5 to 10 fold higher drug level in local administration, as seen in various efficacy, toxicology and PK animal models.

LC400 demonstrates slow and controlled release both in-vitro and in-vivo and provides long-lasting profound analgesia with a minimal risk of adverse effects, due to the low blood concentration of bupivacaine and it will reduce to a minimum the need for systemic opioids.

SIMULATION OF BILAYER SYSTEMS BASED ON NEUTRAL AND CATIONIC LIPIDS IN COMBINATION WITH FLUORESCENT LIPIDS

Dafna Knani
Biotechnology engineering, Braude College, Israel

Liposomes mediate highly effective fusion processes with living cell membranes and therefore can be used for delivery of biomolecules into membranes of living cells as well as cell surface modifications. Recently, a new, simple, and almost universal fusogenic liposome system containing neutral and positively charged lipid molecules, as well as an additional lipid component with aromatic molecular groups was described. As fusion mechanism, a synergistic interaction of the positively charged lipids and the delocalized electron system of the aromatic group inducing local dipoles and membrane instabilities was hypothesized. However, the exact mechanism is not yet understood.

The goal of the present study is to computationaly simulate the bilayer system and the influence of introducing the aromatic lipids into the bilayer. The system was studied by atomistic dynamic simulation of 3D periodic boundary cubic cells of the lipids as well as of bilayer solvated with two layers of water molecules on each side. In addition, Coarse Grained model of the bilayer system was constructed and subjected to dynamic simulation. According to the computational results it seems that the introduction of the aromatic lipid into the bilayer disturbs the bilayer structure and makes it less stable, which facilitates its fusion with the cell membrane.

QUANTITATIVE ANALYSIS OF DRUG DELIVERY TO THE BRAIN VIA NASAL ROUTE

Luba Kozlovskaya, Mohammed Abou-Kaoud, **David Stepensky**
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel

Introduction: The blood-brain barrier (BBB) prevents drugs' permeability into the brain and limits management of brain diseases. Intranasal delivery is a convenient route of drug administration that can bypass the BBB and lead to a direct delivery of the drug to the brain via olfactory and trigeminal pathways. Indeed, drug accumulation in the brain following intranasal application of drug solution, or of drug encapsulated in specialized delivery systems (DDSs), has been reported in numerous scientific publications.

Objectives: to analyze the available data on drug delivery to the brain via nasal route and to reveal the efficiency of drug brain targeting by different types of DDSs.

Methods: We searched for scientific publications published in 1970-2014 that reported delivery of drugs or model compounds to the brain via intranasal and parenteral routes, and contained quantitative data that were sufficient for calculation of brain targeting efficiency. We identified 73 publications that matched the search criteria and analyzed their experimental settings, formulation types, analytical methods, and the claimed efficiencies of drug brain targeting: drug targeting efficiency (%DTE) and nose-to-brain direct transport (%DTP).

Results: Outcomes of this analysis indicate that the efficiency of brain delivery by nasal route differs widely between the studies, and does not correlate with the drug's physicochemical properties. Particle- and gel-based DDSs offer limited advantage for brain drug delivery in comparison to the intranasal administration of drug solution. Nevertheless, incorporation of specialized reagents (e.g., absorption enhancers, mucoadhesive compounds, targeting residues, etc.) can increase the efficiency of drug delivery to the brain via nasal route.

Conclusions: More elaborate and detailed methodological and analytical characterization and standardized reporting of the experimental outcomes are required for reliable quantification of drug targeting to the brain by nasal route. Quantitative analysis of these data will facilitate development of DDSs with high brain targeting efficiency.

NON-INVASIVE INTRAVITAL MONITORING OF DRUG RELEASE FROM NOVEL POLYMERIC THERANOSTIC NANOMEDICINES

Rachel Blau[1], Hemda Baabur-Cohen[1], Shiran Ferber[1], Yana Epshtein[1], Orit Redy-Keisar[2], Einat Kisin-Finfer[2], Doron Shabat[2], Ronit Satchi-Fainaro[1]
[1]*Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel*
[2]*School of Chemistry, Tel Aviv University, Israel*

Theranostics is a relatively new term, introduced in 2002 that describes any material for applications combining both ***thera***py and diag***nostics***. The great challenge for future personalized therapy in oncology is exploring improved methodology for (i) early detection of localized and disseminated tumor cells in patients and (ii) monitoring drug release at the target site in order to evaluate the treatment's efficacy. The determination of both is critical to success of cancer therapy and improvement of patients' survival rates. A theranostic nanosystem composed of nanocarrier, drug and Turn-ON probe is an ideal platform to address these challenges. In this study, we designed, synthesized and characterized a theranostic nanomedicine based on*N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer. The diagnostic system consists of self-quenched Cy5 and the therapeutic system is based on the anticancer agent paclitaxel (PTX). Both diagnostic and therapeutic moieties were conjugated to HPMA copolymer through a Gly-Phe-Leu-Gly (GFLG) linker, cleaved by cathepsin B, a lysosomal cysteine protease overexpressed in several tumor types such as lung, colon, prostate, melanoma and breast cancers. Our systems enable site-specific release of the drug concomitantly with the fluorophore activation to its Turn-ON state upon enzymatic degradation. HPMA copolymer-PTX conjugate inhibited the proliferation of breast cancer cells. Furthermore, our conjugate demonstrated anti-angiogenic properties inhibiting endothelial cells proliferation. Our preliminary results with the diagnostic nano-conjugate HPMA copolymer-Cy5 present its potential use as a novel probe for sensing real-time drug release from the polymeric nanocarrier. This approach of co-delivery of two complementary systems serves as a proof-of-concept for non-invasive real-time deep tissue intravital orthotopic monitoring that may potentially be exploited as a theranostic nanomedicine in the clinic.

GAGOMERS AS CISPLATIN CARRIERS FOR THERAPEUTIC INTERVENTION IN LUNG CANCER

Nizan Cauzmer[1,2,3], Meir Goldsmith[1,2,3], Michael Gozin[4], Michael Gozin, Dan Peer[1,2,3]

[1]*Center for Nanoscience and Nanotechnology, Tel Aviv University, Israel*

[2]*Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel*

[3]*Department of Materials Sciences and Engineering, Faculty of Engineering, Tel Aviv University, Israel*

[4]*School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, Israel*

Cisplatin is the first member of a class of platinum-containing anti-cancer drugs that was approved by FDA for a variety of cancers in 1978 and is a widely used today as a cytotoxic agent for the treatment of bladder, ovarian, testicular, cervical, head and neck, and non-small cell lung cancer. Upon entry to the cell, the cisplatin molecule undergoes hydrolysis, in which a chlorine ligand is replaced by a molecule of water (termed aquation), generating a positively charged species. The resulting hydrolysis product is believed to be the active species, interacting with nucleophilic molecules including DNA, which ultimately triggers apoptosis (programmed cell death).

As with all chemotherapies, cisplatin, distribute throughout the body is also toxic to healthy cells with noted severe side effects including renal toxicity, gastrointestinal toxicity, nephrotoxicity, ototoxicity, and optic neuropathy which limits its use in the clinic. To address the toxic effects and improve the therapeutic outcome, we devised a novel strategy to entrap small molecule drugs in a safer carrier system, which will also consist of a built-in targeting agent towards cancer cells. This carrier is made from naturally-occurring biomaterials that include phospholipids that form lipid particle clusters. Coated with the glycosaminoglycan, hyaluronan (HA) – these new carriers were termed "Gagomers" (GAGs). HA is the major ligand of CD44, which is expressed on almost all cell types, but is up regulated and undergone different splice variations in cancerous cells. The high CD44 expression of various tumors could be used for targeting by GAGs.

INJECTABLE PORCINE CARDIAC EXTRACELLULAR MATRIX AS A CELL PLATFORM FOR MYOCARDIAL REGENERATION

Yael Efraim, Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion – Israel Institute of Technology (IIT), Israel

Cardiovascular diseases continue to be the leading cause of death in the western world, calling for new treatments for progressive heart failure, post myocardial infarction. While acellular and cellular cardiac patches are applied surgically to the epicardial surface of the heart, injectable materials offers the prospective advantage of minimally invasive, delivery directly into the myocardium. We have developed a unique heart based gel that can serve as an injectable scaffold and can be combined with cells, such as human induced pluripotent stem cells (hiPSC) or mesenchymal stem cells, for myocardium rejuvenation. Gels were prepared by solubilizing decellularized porcine cardiac extracellular matrix (pcECM) and studied in combination with polymers such as chitosan and genipin. The gels were characterized by scanning electron microscopy, and mass spectrometery and were found to be mostly made up of collagen (more than 90%) and assembled into thin fibers' mesh (down to 5nm width). The mechanical properties of the gels were measured, showing the gels' strength was elevated by increasing chitosan and genipin concentration. We also evaluated the immunogenic potential of the gel by measuring nitric oxide secretion and TNF-α, IL-1β secretion by qPCR from macrophage cells exposed to lyophilized gel. The gel was non immunogenic. Cells were cultivated within the gels and their viability, proliferation, ECM remodeling and differentiation towards cardiac lineages were determined using the AlamarBlue assay, qPCR and Immunofluorescent staining. The gels supported cell growth and induced expression of cardiac markers. To conclude, injectable ECM-based materials offer minimally invasive cells delivery platform to the heart. Furthermore the unique ability to cultivate hiPSC and their derivative within such construct may substantially contribute to the cardiac engineering field of personalized medicine.

CD44-TARGETED POLYION COMPLEXES FOR siRNA DELIVERY

Moran Golan, Ayelet David
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel

In recent years, siRNA has emerged as an effective technology for gene silencing in a variety of life-threatening diseases. Numerous approaches were developed to generate cell or tissue specific delivery systems of siRNA to be used in vivo, with lipoplexes and polyplexes currently being the most commonly applied. CD44 is a transmembrane glycoprotein expressed by many cell types. Elevated levels of CD44 are associated with a wide variety of tumors and metastasis. Our aim was to design a delivery system which will enable the specific targeting and uptake of siRNA into CD44 overexpressing cancer cells. Thus, we have synthesized polyethylene glycol-block-polyethylene imine (PEG-b-PEI) copolymers that were installed with short peptide sequences derived from the α5 chain of laminin molecule, designated as mA5G27F that can bind to CD44 via its GAGs side chain. The targeted block copolymers demonstrated good in vitro complexation with siRAC1, had lower surface charge relative to their building block, demonstrated stable particle diameter 200 nm, and exhibited low cytotoxicity towards cells compared to the cationic precursor polymer. The in vitro uptake of the targeted-copolymers was significantly higher compared to PEI or PEG-b-PEI in CD44-overexpressing cells compared to the CD44-null cells, HEK293. Moreover, CD44-targeted polyplexes demonstrated high and selective transfection efficiency as evident by the significant inhibition in RAC1 expression in A549 cell. The synthesized targeted polyion complexes demonstrated high stability in human serum, validating once more their suitability for in-vivo use. A Maximum Tolerated Dose (MTD) study in Balb/c mice demonstrated safety of the formulation up to 40 mg/kg for Intrapritoneal administration. These results indicate that mA5G27F-PEG-b-PEI can efficiently deliver siRNA into CD44-overexpressing cells, and the siRNA can reach the perinuclear region for effective gene silencing. The gene silencing potency of mA5G27F-PEG-b-PEI is currently being tested in vivo.

BIO-SURGERY

Assaf Zinger, Chen Tzror, Matan Alper, Avishay Herman, Avi Schroeder
Chemical Engineering, Technion – Israel Institute of Technology, Israel

Blades are the most common surgical tools. However, while approaching a target site the blade may damage healthy tissue, resulting in lengthened recovery and increased pain. Enzymes are the biological means for cleaving tissue in a molecularly-precise manner. In this study we utilized collagenase, loaded into micro- or nano-particles, as a biological scalpel for performing a medical task – cleaving excess collagen. The particles protected the enzyme from degradation, confined its spatial biodistribution, and maintained a therapeutic dose at the treatment site. The in vivo model was aimed at replacing an ordinary surgical procedure in the oral cavity – specifically, relaxing the collagen fibers that connect between the tooth and the underlying bone. After inserting the particles into the gingival sulcus, enhanced orthodontic motion was recorded, due to relaxation (but not cleavage) of the supra-crestal collagen fibers. The recovery of collagen fibers post enzymatic surgery is rapid in comparison to the recovery after ordinary, scalpel-based, oral surgery.This approach, in which drug delivery systems perform a corrective medical task, may increase surgical precision and improve care.

NEOADJUVANT TREATMENT FOR PREVENTION OF BREAST CANCER METASTASIS DEVELOPMENT: αvβ3 INTEGRIN-TARGETED PGA-PACLITAXEL NANOCUNJUGATE

Anat Eldar-Boock[1], Joaqiun Sanchis[2], Ruth Lupu[3], Maria J. Vicent[2], Ronit Satchi-Fainaro[1]

[1]*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel*

[2]*Polymer Therapeutics Laboratory, Centro de Investigación Príncipe Felipe, Spain*

[3]*Mayo Medical Laboratories, Mayo Clinic, USA*

Prevention of metastasis growth presents an unmet clinical need. Anti-angiogenic therapy might provide an alternative way to manipulate cancer, yet it did not materialize into clinical practice. Therefore, combination of anti-angiogenic therapy with cytotoxic therapy directed to the metastatic cancer cells, offers a promising therapeutic approach. Paclitaxel (PTX) is a widely-used potent cytotoxic drug, which also exhibits anti-angiogenic activity at low doses. However, its use is limited by severe side effects, caused by the hydrophobic drug and its solubilizing agents.

We designed and synthesized a novel polyglutamic acid (PGA)-PTX-E-[c(RGDfK)2] nano-sized conjugate. Polymer conjugation converted PTX to a water-soluble macromolecule, which passively targeted the tumor tissue exploiting the enhanced permeability and retention (EPR) effect, while extravasating via the leaky tumor neovasculature. PGA is enzymatically-degradable by cathepsin B, leading to PTX release. The E-[c(RGDfK)2] serves as an additional active targeting to αvβ3 integrin. Integrins play a key role in cell matrix interactions. The highly restricted integrin αvβ3 is overexpressed on tumor endothelial and some epithelial cells, during tumor growth, invasion, and metastasis. PGA-PTX-E-[c(RGDfK)2] displayed a potent anti-angiogenic therapy. Mice bearing orthotopic mammary tumors demonstrated preferential tumor accumulation of the RGD-bearing conjugate, leading to enhanced antitumor efficacy and a marked decrease in toxicity compared with free PTX[1].

We developed a mouse model that mimics the clinical setting, of mammary cancer metastases following resection of the primary tumor. Integrin αvβ3 expression was detected on mCherry-labeled MDA-MB-231 mammary adenocarcinoma cells while circulating in the bloodstream of mice. Using this model, PGA-PTX-E-[c(RGDfK)2] conjugate prevented breast cancer metastases formation following surgical removal of the primary tumor.

Taken together, our conjugate alters the pharmacokinetics of free PTX. Inclusion of an active targeting moiety to integrin expressing-cells, have the potential to prevent breast cancer metastasis development as an anti-angiogenic and anticancer adjuvant therapy.

1. Eldar-Boock et al. Biomaterials. 2011;32(15):3862-74.

ENZYME-RESPONSIVE AMPHIPHILIC PEG-DENDRON HYBRIDS AND THEIR ASSEMBLY INTO SMART MICELLAR NANOCARRIERS

Assaf Harnoy, Ido Rosenbaum, Roey Amir
School of Chemistry, Tel Aviv University, Israel

Stimuli-responsive amphiphilic block copolymers, capable of self-assembling in aqueous media into nano-sized micellar structures, are of great promise in various biomedical applications, including controlled drug delivery and molecular bio-sensing. Utilizing enzymes as stimuli to induce the disassembly of micelles can be considerably advantageous due to the common occurring of enzymatic imbalances, which are associated with many diseases. Furthermore, the high specificity of enzymes towards their substrates, along with their catalytic efficiency, offer additional advantages over other types of induced stimuli (e. g. pH, temperature). We have recently reported a highly modular and novel design for the efficient and simple synthesis of amphiphilic block copolymers based on a linear hydrophilic poly(ethylene glycol) and an enzyme-responsive hydrophobic dendron. These hybrids were shown to self-assemble in aqueous media into spherical nano-containers, which can disassemble and release their encapsulated molecular cargo upon enzymatic stimulation. The obtained results demonstrated that the disassembly rate of the formed micelles can be easily tuned by the length of the polymeric chain. Hence, its adjustment may allow control over the release rate of the encapsulated guest molecules. Such smart amphiphilic hybrids could potentially be applied for the fabrication of nano-carriers with adjustable release rates for controlled drug delivery applications.

REGULATOTRY HIGHLIGHTS OF THE LONG AND COMPLICATED PATH FROM THE BENCH TO THE MARKET

Chaya Mazouz[1], Doron Friedman[1], Liza Silverman[2], Dima Shamrakov[1], Yanir Aldouby[1], Tal Berman[2], David Stepensky[3], Chezy Barenholz[1,2]

[1]*Lipocure, Ltd., Israel*

[2]*Biochemistry, Hebrew University - Hadassah Medical School, Israel*

[3]*Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel*

QbD is a systematic methodology for both innovative and generic pharmaceutical development. It begins with predefined objectives and emphasizes product, process and process control understanding, based on sound science and quality risk management. It means designing and developing formulations and manufacturing processes to ensure a predefined therapeutic product (Yu et al. 2009).

The liposomal product, being a supramolacular assembly, represents a very complex case due to the many possibilities to vary the composition and physicochemical properties (Jiang et al., 2011); therefore QbD should be followed (Lionberger et al., 2008).

For a generic product, the quality target product profile (QTPP) is defined based on the properties of the drug substance and the required performance. The next step is to design the formulation and identify the critical quality attributes of the final product that must be controlled to meet the QTPP. Scientific and published information available combined with reverse engineering of similar products provides the bases for the development process. Risk assessment should be executed at various time points in order to prioritize knowledge gaps for further investigation of the manufacturing process, raw materials, in-process controls and final release parameters.

Finally, we were able to establish a robust process allowing the manufacturing of a well-defined and safe product, while continually monitoring and updating the process to assure consistent quality.

Furthermore, it's important to understand the relationship between *in vitro* and *in vivo* performance, e.g. *in vitro in vivo* correlation (IVIVC) in order to evaluate the impact of formulation and process variable changes on drug product quality during development and to facilitate the evaluation of post-approval changes. Therefore, we decided to develop a predictive dissolution (drug release) method that is relevant to the in vivo situation in order to establish IVIVC.

CELL DERIVED NANO-GHOSTS AS A NEW GENE DELIVERY SYSTEM FOR CANCER THERAPY

Limor Kaneti, Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion, Israel

We aim to develop a novel targeted non-viral gene delivery system for cancer therapy, which is based on unique vesicles produced from the cell membrane of mesenchymal stem cells (MSC). MSC are known for their homing capability towards cancer cells and their microenvironment. These "Nano-Ghosts" (NG) will benefit the surface molecules of the MSC and thus may preserve their targeting mechanism. Into this "Trojan horse" NG system we will incorporate therapeutic proteins encoding DNA, which will be expressed at the tumor site.

NG preparation resulted in round vesicles with an average size of 180nm and a zeta potential of about -16mV. FACS analysis demonstrated preservation of most of the original MSC surface markers. NG-PC3 (prostate cancer cells) interaction was evaluated by FACS to show specific and time-dependent accumulation inside the cytoplasm and nucleus. pDNA was successfully incorporated to the NG system. The transfection capability to PC3 cells was assessed, by using a PEX (anti-cancerous protein- C-terminal hemopexin-like domain of MMP2) encoding plasmid and observing its entrance to the cells and expression using real-time PCR and Alamar blue/Annexin-PI. Results demonstrated significant apoptosis and a decline in cell viability and proliferation following a 6 hr incubation with NG-pPEX.

Finally, In vivo studies were conducted. Athymic nude mice were inoculated with PC3 tumors. When the tumors reached $100mm^3$- treatments were injected; IV and IP injected NG-pPEX were compared to empty NG and naked DNA as well as to untreated mice. A significant inhibition in tumor growth was achieved after a single administration of NG-pPEX compared to controls. Tumors were sectioned, stained and analyzed for CD31, ki67 and caspase3 presence, as indicators for vascularity, proliferation and apoptosis, respectively. A significant reduction in tumor vascularization and proliferation indices, as well as an increase in apoptosis index were achieved, in mice treated with NG-pPEX, compared to the controls.

TRACKING INFLAMMATION IN THE EPILEPTOGENIC BRAIN TISSUE IN THE RAT BY MULTIFUNCTIONAL NANOPARTICLES

Emma Portnoy[1,4], Dorrit Inbar[2], Boris Polyak[3], Ahmad Rai[3], Gilad Kenan[2], Ameer Bishara[1], Aniv Mann[1], Gal Itzhak[1], Shlomo Magdassi[4], Dana Ekstein[2], Sara Eyal[1]

[1]*Institute for drug research, Hebrew university, Israel*

[2]*Department of Neurology, Hadassah, Hebrew university, Israel*

[3]*Department of Surgery, Drexel university, USA*

[4]*Casali Institute, Hebrew university, Israel*

Accumulating evidence suggests a positive feedback cycle between brain inflammation and epileptogenesis. Recurrent seizures lead to activated immune responses which in turn further increase neuronal excitability and induce BBB breakdown. In this study, we utilized biocompatible nanoparticles (NP) detectible by optical imaging to target activated macrophage-like cells in epileptogenic brain tissue, taking advantage of local immune cell activation and BBB disruption.

Polylactic acid (PLA)-based NP were prepared by a modified emulsification-solvent evaporation method with the incorporation of oleate-coated magnetite nanocrystals. In vitro NP uptake was assessed using RAW 246.7 cells. The in vivo cellular NP distribution was evaluated using the lithium-pilocarpine model of temporal lobe epilepsy.

The NP were rapidly uptaken by RAW 246.7 cells, with $T_{1/2}$ of 0.23 ± 0.02 hr and Emax of 99.7% ± 2.6%. In vivo, the NP were detected in the brains of rats with spontaneous seizures, but not in controls. In rats in which spontaneous seizures were induced, the NP accumulated almost exclusively in macrophages/microglia, with greater accumulation in CA1 at 3 hr, 6 hr and 24 hr, compared to 7 days (3.9 ± 3.6, 3.8 ± 2.3 and ± 3.6 ± 2.0 vs. 1.2 ± 1.4 NP/high power field (HPF), respectively; P0.05), but no significant time course differences in the CA3 (0.9 ± 1.3, 0.7 ± 0.7, 1.5 ± 1.1 and 0.9 ± 1.2 NP/HPF at 3 hr 6 hr, 24 hr, and 7 d, respectively).

We established a proof of concept for the ability to deliver NP to macrophages/microglia in epileptogenic brain tissue. Our proposed traceable NP, uptaken by circulating or resident phagocytic cells, could contribute to the proper localization of epileptic foci. In addition, this system may be utilized for drug delivery and contribute to elucidating the immune contribution to the development and persistence of epileptic seizures.

POSTER #25

NANOSWIMMERS FOR TARGETED DRUG DELIVERY

Nour Zoaby, Janna Shainsky, Avi Schroeder
Chemical Eng., Technion, Israel

Penetrating deep cancerous tissues is an unmet need for most parenterally-administered drugs. Several externally-powered drug delivery systems have been developed to address this issue, using magnets or near-infrared light to maneuver therapeutics towards the disease site.

Here we describe an autonomously powered, self-destructing system, for carrying therapeutic nanoparticles to cancer sites. The system is based on motile bacteria loaded with therapeutic nanoparticles. Bacteria have been shown to use chemotaxis to propel towards nutrient-rich cancer sites. There, bacteria can penetrate the cells or remain in the extra-cellular space. Flagella, extending from the bacterial membrane, facilitate motion under low Reinhold's conditions existing in the tumor microenvironment. At the tumor site, the loaded drug, doxorubicin, kills the bacteria, disassembling its membrane and releasing the remaining drug to execute anti-cancer activity.

We present here the velocities of nanoparticle-loaded bacteria under different clinically relevant pH and glucose conditions, and within medium of various viscosities. The uptake of bacteria by breast-cancer cells (4T1) was imaged using confocal microscopy and the ability of the carrier to 'self-destroy' during drug release was demonstrated.The biodistribution of self-propelled bacterial systems to tumors in vivo was tested.

Coupling between the emerging advantages of bacteria-based cancer therapies, and their capacity to act as autonomously propelled, self-destructive, nanomedicine carriers, may grant new clinical capacities.

pfSUB1-ACTIVATED IMMUNOZYMOXINS FOR TREATING MALARIA

Almog Bitton, Itai Benhar
Molecular Microbiology and Biotechnology, Tel Aviv University, Israel

Introduction: The increasing resistance of malaria parasites to antimalarial drugs is a major contributor to the reemergence of the disease and the spread of malaria to new locations and populations. My research group has recently presented protease-activated toxins termed "zymoxins" as a new approach for intervention in diseases where specific proteolysis plays an essential role. In my project I study zymoxins designed to be activated by malarial proteases thereby halting the spread of the parasite in the infected host. My research objectives are to produce the malarial protease PfSUB1 in E. coli; produce PfSUB1-activated zymoxins; and show a proof-of-concept for malarial zymoxins' efficacy in our model cell line PfSUB1-expressing HEK293.

Methods: E. coli BL21 Rosetta (DE3) or SHuffle (K12) cells were used for expression of the T7 promoter-driven recombinant toxins, protease, and cleavable substrates. For purification HisTrap or MBP-Trap (amylose) columns were used. In vitro PfSUB1 mediated cleavage products were verified by western blot. HEK293 stably expressing the tetracycline repressor protein (T-REx) were transfected with X-tremeGENE 9 reagent. The cell-killing activities of zymoxins are measured by an MTT assay.

Results: Two zymoxin constructs are evaluated; "PE-DTA-cleavage site-defensin" and "PE-Ricin A-cleavage site-stalk peptide". In these constructs, defensin and stalk peptide are the inhibitors of DTA and RTA respectively, while PE (Pseudomonas exotoxin A) is used as a delivery platform for binding and translocation. Successful in vitro cleavage results of EGFP-cleavage-site-CBD and zymoxins were shown with insect cells' derived rPfSUB1 (Withers-Martinez et al., 2002), since various attempts to produce an active form of rPfSUB1 in E. coli have failed. HEK293 cells were stably transfected with EGFP-PfSUB1 followed by FACS to obtain high PfSUB1 expressing cells. Viability assays for HEK293PfSUB1+ cells in the presence of malarial zymoxins are designed to show a proof-of-concept for the eradication of parasitemia by PfSUB1-activated zymoxins.

FLUORESCENT POLYSTYRENE/POLYSTYRYLBISPHOSPHONATE CORE SHELL MICROSPHERES – A POTENTIAL BONE CEMENT COMPONENT

Ravit Chen[1], Jenny Goldman[2], Shlomo Margel[2]
[1]*Organic Chemistry, Israel Institute for Biological Research, Israel*
[2]*Chemistry, Institute of Nanotechnology and Advanced Materials, Israel*

Bisphosphonates (BPs) are chemically stable analogues of inorganic pyrophosphates possessing two phosphonate groups linked to a single carbon (P-C-P). Their ability to form bidentate or tridentate chelates with calcium ions results in a higher affinity to hydroxyapatite (HAP).[1] Consequently, BPs form stronger interactions with dentin, enamel and bone. Recently, Styrylbisphosphonate (StBP) non-biodegradable vinylic monomer was synthesized in our laboratory and presented high affinity to HAP.[2]

In this study, we describe the preparation and characterization of NIR fluorescent Polystyrene/Polystyrylbisphosphonate (PS/PStBP) core shell micrometer sized particles of narrow size distribution. For this purpose, a new hydrophobic NIR fluorescent dye, Benzyl heptamethine cyanine (BHC) was designed and synthesized by condensation of 3–benzyl–1,1,2–trimethyl–1H–benzo[e]indole–3–ium bromide with glutaconaldehyde dianyl monohydrocloride. Polystyrene (PS) microspheres of narrow-size distribution were synthesized by dispersion polymerization of styrene in ethanol and 2-methoxyethanol, according to the literature. The PS particles were activated by mild ozonolysis prior to the encapsulation in order to avoid dye destructing. Encapsulation of the new hydrophobic NIR dye was performed by a single - step swelling process within the oxidized PS microspheres.

Graft polymerization of the StBP monomer onto the entrapped BHC oxidized PS microspheres led to the desired NIR fluorescent PS/PStBP core-shell microspheres. The particles' fluorescence was demonstrated by laser scanning confocal microscopy (LSCFM). The surface bisphosphonic function was evaluated by hydroxyapatite mineralization procedure using characterization methods such as HRSEM, TEM, and EDX. The results indicate that these non-biodegradable Fluorescent PS/PStBP particles can potentially be used as component of bone cement for various orthopedic procedures.

NANOENGINEERING GOLD NANOPARTICLE - COMPOSITE SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING

Michal Shevach[1,2], Ben Maoz[2,3], Ron Feiner[1,2], Assaf Shapira[1], Tal Dvir[1,2]
[1]*Molecular Micro. & Biotechnology, Tel Aviv University, Israel*
[2]*Center for Nanoscience and Nanotechnology, Tel Aviv University, Israel*
[3]*School of Chemistry, Tel Aviv University, Israel*

Cardiovascular diseases are the leading cause of death in the western world. Upon myocardial infarction (MI), a part of cardiac tissue loses its contractile function, which eventually could lead to heart failure and patient's death. The aim of cardiac tissue engineering is to develop functioning 3-dimensional (3D) cardiac patches that would restore heart function after MI. These patches are composed of cardiac cells and biomaterial scaffolds that mimic the native microenvironment of cardiac cells. These scaffolds are usually fabricated from non-conductive materials, limiting the propagation of electrical signal between adjacent cardiac cells. Here we report a simple approach for fabricating 3D gold nanoparticle (NP)-based fibrous scaffolds, for engineering functional cardiac tissues generating a strong contraction force. In a facile method, gold NPs were evaporated on the surface of the fibers, creating nanocomposites with various nominal gold thicknesses. First, we evaluated the structural, chemical, mechanical and electrical properties of the composite scaffolds. Then, we assessed the effect of the composite scaffolds on cardiac cell organization and function. We found that compared to pristine scaffolds, cardiac cells seeded on the nano-gold scaffolds assembled into more elongated and aligned tissues, with significantly higher aspect ratio. The gold NPs on the fibers were able to maintain the ratio of cardiomyocytes to fibroblasts in the culture and promote massive cardiac sarcomeric actinin expression, similar to native cardiac tissue. Finally, engineering cardiac tissues within gold NP-based scaffolds exhibited significantly higher contraction amplitudes, rates and electrical signal propagation velocity, as compared to scaffolds without gold. We are now in the final stages of *in-vivo* experimental testing the ability of the composite scaffolds to improve cardiac function in a rat MI model. We envision that cardiac tissues engineered within these gold NP scaffolds could be used in the future for human care.

POSTERS

MULTI- MODAL DETECTION OF COLON MALIGNANCY BY NIR-TAGGED RECOGNITION POLYMERS AND ULTRASOUND CONTRAST AGENT

Meital Bloch[1], Lauren Jablonowski[2], Dorit Moradov[1], Irena Javsarov[1], Eylon Yavin[1], Abraham Nyska[3], Margaret Wheatley[2], Abraham Rubinstein[1]

[1]*The School of Pharmacy Institute for Drug Research, The Hebrew University of Jerusalem, Faculty of Medicine,, Israel*

[2]*School of Biomedical Engineering, Science & Health Systems, Drexel University, USA*

[3]*Sackler School of Medicine Consultant in toxicologic pathology, Tel Aviv Univertsity, Israel*

Polyps in the colon epithelium are commonly detected by colonoscopy. To increase colonoscopy competence in ambiguous situations and to increase its capability to discriminate benign from malignant polyps, we suggest a combined tactics that associates two approaches to obtain a synergistic effect during diagnostic procedures in real time. Water-soluble cationized polyacrylamide (CPAA), was tagged with NIR dye to form Flu-CPAA. The recognition peptide VRPMPLQ was then conjugated with the polymer to form Flu-CPAA-Pep, which was then incorporated into echogenic microbubbles (MBs) made of polylactic acid. This proof of principle study, which examines the system components discretely, is the first, in a series of experiments aimed at the ultimate design in which, after intravenous administration and upon arrival at the vicinity of a suspected malignant region in the colon, the MBs would break into nanoscale particles, under local US interrogation. This would allow nano scale particles escape from the vasculature, carrying the polymeric cargo which, in turn, binds specifically to the malignant tissue. It is anticipated that he resulting spatial increase in fluorescence will be detected from the aspect of the colon lumen by means of an endoscopic probe equipped with an NIR imager. MBs were examined, in vitro and in vivo, and they were also tested for their capability to break into nano shards (NS) at the focus of an US beam. It was found that VRPMPLQ increased NS specific attachment, especially in the 100- Flu-CPAA-Pep and decreased polymer cytotoxicity. US and NS production, did not interfere with the products specific binding. The in vivo studies supported this observation and the US-driven generation of NS resulted in attachment similar to that of the unloaded polymers. However, the attachment of VRPMPLQ to the polymeric backbone reduced the preferential attachment caused by the polymer charge density.

LIPOSOMAL DELIVERY OF ANTIMICROBIAL PEPTIDES FOR HSV THERAPY

Sapir Ron Doitch[1], Hadar meirov[1], Mirjam Nordling David[1], Ina Hogk[2], Steffen Rupp[2], Gershon Golomb[1]

[1]*School of Pharmacy, Faculty of Medicine,, Institute for Drug Research, The Hebrew University of Jerusalem, Israel*

[2]*Biotechnology, Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany*

Over 90 % of the world population is infected with herpes simplex virus (HSV) which causes the most common skin disease. HSV-1 and -2 persist in the body by becoming latent and hiding from the immune system in the cell bodies of neurons. Consequently, herpes viruses establish lifelong infections and the virus cannot currently be eradicated from the body. Hence, to date, there are no effective cures for HSV infection but rather symptomatic treatment only. Antimicrobial peptides (also: cationic host defense peptides) are short, positively charged amino-acid sequences which acquire alpha-helical shapes upon biological membrane interaction thus interrupting its integrity. These peptides are naturally occurring in innate immunity cells of various organisms and are active against a wide range of pathogenic microorganisms. However, both peptides are also highly toxic to eukaryotic cells.

A liposomal formulation containing antimicrobial peptide (Indolicidin or LL37) and targeted to specific sites characteristic with HSV latent infection would allow the protection of these peptides, reduce adverse effects and would concentrate the antiviral activity to HSV infected cells, towards an effective cure. We have developed PEGylated ("stealth") liposomal formulations containing Indolicidin which have shown to be significantly less toxic *in vitro* compared to the naked peptide, and FITC-labeled-LL37 formulations that have shown to be successfully uptake into naïve eukaryotic cells. The Rupp group at FhG-IGB has established a novel *in vitro* HSV-1 infection model which is modified by integrating a latently infected neuronal cell line within the dermal layer of the 3D skin equivalent. Its main characteristic is a specific reactivation of HSV-1, which can be achieved by stress conditions. In ongoing studies the toxicity and anti-HSV activity of both peptides are evaluated and a liposomal formulation of non-labeled LL37 is characterized and optimized.

BETA-CASEIN BASED NANO-VEHICLES FOR ORAL DELIVERY OF CHEMOTHERAPEUTIC COMBINATIONS TO OVERCOME MULTIDRUG RESISTANCE IN GASTRIC CANCER

Maya Bar-Zeev[1,2], Yehuda G. Assaraf[3], Yoav D. Livney[1,2]
[1]*Russell Berrie Nanotechnology Institute, Technion - Israel Institute of Technology, Israel*
[2]*Laboratory of Food Physical Chemistry and biopolymeric delivery systems for health, Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Israel*
[3]*The Fred Wyszkowski Cancer Research Laboratory, Department of Biology, Technion - Israel Institute of Technology, Israel*

Multidrug resistance (MDR) is a major hurdle toward curative chemotherapy. Furthermore, the vast majority of chemotherapeutic agents is lipid-soluble, administered intravenously using harmful solvents and surfactants. The availability of effective and selective oral delivery system would significantly contribute to patients' quality of life and save hospitalization costs. Bovine β-casein (β-CN) is an abundant milk protein that has a pronounced amphiphilic structure[1], promoting it's self-assembly to stable micelles in aqueous solutions. β-CN contains 17% proline residues, leading to an open tertiary structure[1] which is easily accessible to gastric proteases. In previous studies we introduced the potential of β-CN micelles as oral delivery vehicles for the target-activated release of hydrophobic bio-actives cargo, including nutraceuticals and drugs, such as chemotherapeutic agents[2] for gastric cancer treatment[3]. In the current research we explore the possibility to use different combinations of encapsulated hydrophobic anticancer drugs (e.g. Paclitaxel, Mitoxantrone) along with their corresponding encapsulated hydrophobic chemo-sensitizers (e.g. Tariquidar, Ko143 respectively), which counteract MDR mechanisms, that expel a spectrum of anticancer drugs from cancer cells, based on ATP-driven MDR efflux pumps (e.g. P-glycoprotein/ ABCB1, breast cancer resistance protein/ ABCG2). Hence, the rationally designed encapsulated pair is expected to display enhanced efficacy and synergy in the overcoming of MDR phenomena in gastric cancer. This novel treatment strategy will significantly promote patient compliance as it would not require medical assistance or equipment, thereby avoiding the need for multiple hospitalizations and enable treatment at the comfort of the patient's home.

KEYWORDS

β-casein micelles, Oral chemotherapy, Multidrug resistance.

REFERENCES

1. Y.D. Livney, A.L. Schwan, D.G. Dalgleish, Journal of Dairy Science 2004, 87, 3638-3647.
2. A. Shapira, Y.G. Assaraf, Y.D. Livney, Nanomedicine: Nanotechnology, Biology and Medicine 2010, 6, 119-126.
3. A. Shapira, I. Davidson, N. Avni, Y.G. Assaraf, Y.D. Livney, European Journal of Pharmaceutics and Biopharmaceutics 2012, 80, 298-305.

ELECTRICAL STIMULATION AND MECHANICAL TENSILE INTEGRATED INTO A PERFUSION-BIOREACTOR FOR APPROVING RESEEDING OF DECELLULARIZED CARDIAC PORCINE ECM

Maskit Gvirtz, Marcelle Machluf
Biotechnology and food engineering, Technion, Israel

The successful reseeding of tissue-engineered constructs, with regenerative cells, remains a substantial obstacle in achieving a functional tissue; especially when dealing with complex and thick cardiac constructs, such as decellularized porcine cardiac ECM (pcECM) that is largely investigated by our group. As such, any bioreactor system intended to perfuse and feed the pcECM should necessarily provide mechanical and electrical stimuli, which have been shown to support and direct cell growth, differentiation and the overall tissue functionality. A computerized system was designed to generate electrical stimuli in the form of cardiac action potential shaped waveform, synchronized with an inflating deflating balloon placed beneath the ECM scaffold applying movement mimicking the cardiac volume changes. Both incorporated into a perfusion bioreactor that was previously reported by us in order to support the survival of mesenchymal stem cells, endothelial cells and cardiomyocytes reseeded onto decellularized pcECM. Seeding umbilical cord mesenchymal stem cells (UcMSCs) on the pcECM and applying a gradual rise in perfusion rate, combined with an increasing rhythm of mechanical and electrical stimulations showed initial results of significant increase in viability and cell growth. Future applications of this system, include culturing pcECM scaffolds with regenerative cells and assessing the effect of the various stimuli on cell growth, differentiation and ECM remodeling by various single and co-cultures.

MIRNA-BASED NANOMEDICINE FOR THE TREATMENT OF GLIOBLASTOMA

Paula Ofek[1], Marcelo Calderon[2], Fatemeh Sheikhi-Mehrabadi[2], Shiran Ferber[1], Rainer Haag[2], Ronit Satchi-Fainaro[1]

[1]*Physiology and Pharmacology, Tel Aviv University, Israel*

[2]*Organic and Macromolecular Chemistry, Freie University of Berlin, Germany*

Glioblastoma multiforme (GBM) is an aggressive primary neoplasm of the brain that exhibits notable refractivity to standard treatment regimens. Recent large-scale molecular profiling has revealed deregulated molecular networks as potential targets for therapeutic development. MicroRNA-34a (miR-34a) is downregulated in several tumors including glioblastoma, we therefore aim to evaluate its therapeutic potential for GBM.

Nevertheless, *in vivo* delivery of small interfering RNA (siRNA) and miRNA remains a crucial challenge for their therapeutic success. siRNAs and miRNAs on their own are not taken-up by most mammalian cells in a way that preserves their activity. In order to circumvent these limitations, we developed a cationic carrier system, which can strongly improve their stability, intracellular trafficking and silencing efficiency. Polyglycerol-Amine (PG-NH_2), a water-soluble polyglycerol-based hyperbranched dendritic polymer accumulates in the tumor microenvironment due to the enhanced permeability and retention (EPR) effect, and therefore, represents an ideal nanocarrier for antitumor biological agents.

Using our novel positively-charged nanocarrier, we have studied the expression targets and functional effects of miR-34a in several human glioblastoma cell lines and human tissue samples. miR-34a levels inversely correlated to their target gene levels measured in the same cell lines or tissue. Transient transfection of PG-NH_2-miR-34a polyplex into glioblastoma cells strongly inhibited cell proliferation, cell cycle progression, and cell migration. Consequently, we performed an *in vivo* experiment and achieved a significant tumor growth inhibition following treatment with PG-NH_2-miR-34a polyplex in a human glioblastoma mouse model.

We further developed and evaluated new derivatives of PG-NH_2 that improved its performance in terms of tumor accumulation, intracellular trafficking and microRNA activity. Together, our findings suggest that our polyplex could serve as a potential nanomedicine for glioblastoma.

REFERENCES

- Tiram G. et al., Journal of Biomedical Nanotechnology, 10, 50-66 (2014).
- Ofek P. et al., *The FASEB Journal*, 24(9), 3122-34 (2010).

LOCAL SUSTAINED-RELEASE DELIVERY SYSTEMS OF ANTI-BIOFILM AGENTS FOR PREVENTION OF CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

Julia Shenderovich[1], Doron Steinberg[2], Eran Lavy[3], Michael Friedman[1]
[1]*The Institute for Drug Research, The Hebrew University of Jerusalem, Israel*
[2]*The Faculty of Dental Medicine, The Hebrew University of Jerusalem, Israel*
[3]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel*

Catheter-associated urinary tract infections (CAUTI's) are the most common infections inside hospitals and a major cause of morbidity and mortality. The microorganisms involved in these infections create a biofilm on the surface of the catheter, thus becoming more virulent and less sensitive to antimicrobial agents. Current treatments are associated with low efficacy and high costs. It is known that microorganisms inside the biofilm communicate between themselves in a process called quorum sensing. Disruption of this process by anti-quorum sensing agents might prevent catheter biofilms. Antiseptic agents are also potential means of preventing catheter-associated infections. In order to obtain an effective prevention of CAUTI's, the agent has to be present in the site of action as long as the catheter is being used. Local sustained-release applications prolong the residence of the active agent in the target site, while significantly reducing adverse effects. TZD-8 (5-octylidenethiazolidine-2,4-dione), a molecule with anti-quorum sensing properties, and chlorhexidine, an antiseptic, have shown promising activity against biofilm formation. The objectives of this study are to develop sustained-release delivery systems of TZD-8/chlorhexidine, characterize them and evaluate their microbiological activity *in-vitro* and in a dog model. A delivery system in the form of a polymeric varnish was chosen. TZD-8 and chlorhexidine were successfully incorporated into sustained-release delivery systems. The factors affecting the release rate of TZD-8 from the systems were studied. Several TZD-8 varnishes based on ethylcellulose or ammonio – methacrylate copolymer NF (Eudragit® RL) exhibited an optimal release rate of about 48 hours *in-vitro*. Selected systems were proven to be retained on latex and silicone catheter surfaces for prolonged periods. A sustained-release delivery system of chlorhexidine, previously developed in our laboratory, was tested in a dog model. Urinary catheters coated with the delivery system and inserted to dogs led to a reduced biofilm accumulation in comparison to untreated catheters.

TARGETING NCAM-EXPRESSING TUMORS WITH POLYMER THERAPEUTICS

Ela Markovsky, Ronit Satchi-Fainaro
Department of Physiology and Pharmacology, Tel Aviv University, Israel

Neural cell adhesion molecule (NCAM) expression is associated with an aggressive biological behavior, increased metastasis and expression of stem-cell markers in several tumor types [1]. Several neoplasms such as melanoma, neuroblastoma and some ovarian cancers, overexpressed NCAM on the cell surface of the whole cancer cell population. NCAM is also expressed on cancer stem cells (CSC) in Wilms' tumor, providing a specific biomarker than can be exploited to target these cells [2]. CSCs form a specific population within the tumor that has self-renewal and differentiation properties, increased ability to migrate and form metastases, and increased resistance to chemotherapy. Consequently, even a small number of cells remaining after therapy can repopulate the tumor and cause recurrence of the disease. Here, we aim to selectively target NCAM-expressing tumors using a biodegradable polyglutamic acid (PGA) backbone as the polymeric nanocarrier, conjugated to an NCAM-targeting peptide (C3) and paclitaxel (PTX). In addition to actively targeting NCAM, this system targets the tumor passively via the "enhanced permeability and retention (EPR) effect" [3].

Conjugates of PGA with PTX and C3 or control peptide (C3ala) were synthesized and characterized. PGA-C3 was fluorescently-labeled and shown to bind high-NCAM-expressing IMR-32 human neuroblastoma cells more than control PGA-C3ala conjugate. The conjugate exhibited enhanced cytotoxic and anti-migratory effect compared to control PGA-C3ala-PTX and non-targeted PGA-PTX conjugates when treating IMR-32 human neuroblastoma cells. Moreover, it inhibited capillary-like tube formation of endothelial cells. Similar results were obtained for several other NCAM-expressing cancer cell lines. Our next goal is to evaluate the antitumor activity of PGA-PTX-C3 conjugate on mouse xenograft models of tumors expressing high NCAM levels and tumors with NCAM-CSCs.

KEYWORDS

Polymer therapeutics, NCAM, cancer

REFERENCES

1. Zecchini et al. Neurochem Res 2008.
2. Pode-Shakked et al. J Cell Mol Med 2008.
3. Markovsky et al. J Control Release 2012, 161:446-460.

FOLATE RECEPTOR-TARGETED DRUG DELIVERY SYSTEMS OF DOXORUBICIN FOR THE TREATMENT OF CANCER

Anna Scomparin[1], Anat Eldar-Boock[1], Dikla Ben Shushan[1], Shiran Ferber[1], Galia Tiram[1], Hilary Shmeeda[2], Alberto Gabizon[2], Stefano Salmaso[3], Paolo Caliceti[3], Natalie Landa-Rouben[4], Jonathan Leor[4], Ronit Satchi-Fainaro[1]

[1]*Physiology and Pharmacology, Tel Aviv University*

[2]*Department of Oncology, Shaare Zedek Medical Center, Hebrew University*

[3]*Pharmaceutical Sciences, University of Padova*

[4]*Neufeld Cardiac Research Institute, Sheeba Medical Center, Tel Aviv University*

Supramolecular drug carriers are designed to accumulate in tumors by passive targeting via the enhanced permeability and retention (EPR) effect. The conjugation of targeting moieties results in receptor-mediated selective drug delivery. Folic acid (FA) is a low molecular weight ligand of the folate receptor (FR), widely used as targeting agent due to overexpression of FR on many types of cancer cells[1]. In the present work, we compare the in vitro and in vivo activity of two drug delivery systems (DDS) bearing doxorubicin (Dox) as the active drug and FA as the targeting moiety. In the first approach, two pullulan (Pull)-based prodrugs of Dox were synthesized, distinguished by the presence or absence of FA as targeting moiety, namely Pull-PEG-FA-Dox and Pull-PEG-Dox[2]. The second DDS is PEGylated liposomal doxorubicin (PLD, Doxil™) and its folated version (PLD-FA)[3].

The specific binding of Pull-PEG-FA-Dox and PLD-FA to the FR was demonstrated by a competition assay using [^{3}H]FA on folate receptor-overexpressing human cervical carcinoma, KB cells. Treatment of KB cervical carcinoma-bearing mice with the DDS with 3 weekly injections (cumulative 15 mg/kg Dox-equivalent dose) resulted in enhanced anticancer activity of PLD-FA compared to PLD. In a second experiment, where the DDS were administered i.v. every other day with 15 mg/kg Dox-equivalent dose, the folated-Pull conjugate displayed increased activity compared to the non-targeted carrier. Moreover, both DDS were able to abrogate doxorubicin-induced cardiotoxicity. This study constitutes the first side by side comparison of two actively targeted well-established systems, polymer therapeutics versus liposomal systems in the same mouse tumor model evaluated in parallel.

REFERENCES

1 Gabizon, A., et al. in Targeted Drug Strategies for Cancer and Inflammation, Eds. Leamon & Jackman, Springer-Verlag, Heidelberg, Germany, p. 217-247 (2012).

2 Scomparin, A., et a. Eur J Pharm Sci 42, 547-558 (2011).

3 Gabizon, A., et al. Bioconjug Chem 10, 289-298 (1999).

POLYMERIC NANOPARTICLES OF RAPAMYCIN FOR BREAST CANCER TREATMENT

Muntimadugu Eameema[1], Katiyar Sameer S[1], Domb Abraham J[2], Khan Wahid[1]
[1]*Pharmaceutics, NIPER-Hyderabad, India*
[2]*School of Pharmacy-Faculty of Medicine, Hebrew University of Jerusalem, Israel*

Rapamycin (RPM) exhibits several seemingly unrelated properties such as antibacterial activity, antifungal (anti-candida), and immunosuppressive effects.Implications of RPM for breast cancer are also well reported as it inhibits mTOR (mammalian Target of Rapamycin) signalling pathway. Like most of the anti cancer drugs, P-gp efflux prevents RPM from reaching the site of action. This could be one of the major causes for tumor cells to acquire multidrug resistance (MDR).Less than 20% oral bioavailability and decreased efficacy due to P-gp efflux limit the use of RPM. The present study focused on designing an efficient delivery system for RPM. PLGA as a drug carrier moderates the P-gp eff ect and MDR reversal activity. Hence, PLGA nanoparticles were selected for RPM delivery. RPM loaded nanoparticles were prepared by nanoprecipitation method. The obtained nanoparticles were in the size range of 151.65±19.73, with 72.61±1.13% RPM encapsulation efficiency. In vitro release studies were carried out and 76% of RPM release was observed in 49 days. Further, everted gut sac method was used to study the effect of p-gp efflux on RPM transport and intestinal permeability study was conducted to understand the potential of polymeric nanoparticles in oral absorption. Results indicated that cellular uptake of RPM was increased from nanoparticles owing to moderate P-gp inhibition by PLGA. Pharamacokinetics further supported improved bioavailability of RPM from polymeric nanoparticles than free drug suspension. Efficacy studies are in progress, results so far suggest that use of PLGA nanoparticles would be a promising approach in the treatment of multidrug resistant cancer.

HARVESTING LOW MOLECULAR WEIGHT BIOMARKERS USING GOLD NANOPARTICLES

Luai Khoury[1], Riki Goldbart[2], Tamar Traitel[2], Joseph Kost[1,2], Giora Enden[1]
[1]*Biomedical Engineering, Ben-Gurion University of the Negev, Israel*
[2]*Chemical Engineering, Ben-Gurion University of the Negev, Israel*

The efficacy of cancer treatment depends on its pathological progress: the earlier the detection and diagnosis, the more efficient is the treatment. Several methods are used to detect cancer, such as Computed Axial Tomography (CAT) scan, ultrasound, and biopsy. Deficiencies in the aforementioned methods have stimulated pursuits for alternative detection and diagnostic approaches. One of the most promising approaches is based on the fact that tumor cells excrete low molecular weight (LMW) bio-molecules [1].

In this research we developed and characterized a biomarker harvesting platform based on layer by layer (LBL) coating of gold nanoparticles, first with positively charged poly (ethyleneimine) (PEI) followed by poly (acrylic acid) (PAA).The resulting nanoparticles are 14.5± 2.5 nm in diameter and ζ –potential of -53.84.2 mV. We chose the Stromal Derived Factor alpha protein (SDFα) biomarker as a test case because of its challenging properties: its extremely low concentration (~1.5ng/mL) and its short half-life time in the blood [2]. PAA-PEI-Au (PPAu) nanoparticles exhibit excellent binding specificity, by selectively harvesting the positively charged LMW SDFα (MW=8 kDa, pI=10.3) from serum containing abundant proteins, such as bovine serum albumin, IgG and haptoglobin. This systemcan be used as a platform for various bio-applications such as separating LMW proteins and sensing specific disease biomarkers.

REFERENCES

1. Geho, D. H.; Jones, C. D.; Petricoin, E. F.; Liotta, L. A. *Curr. Opin. Chem. Biol.* 2006*, 1,* 56-61.

2. Vicari, A. P.; Caux, C. *Cytokine Growth Factor Rev.* 2002*, 2,* 143-154.

NOVEL SIROLIMUS LOCAL ORAL SUSAINED RELEASE DELIVERY SYSTEMS FOR PREVENTION AND TREATMENT OF ORAL CANCER

Zakhar Nudelman[1], Michael Friedman[1], Mordechai Findler[2], Anna Pikovsky[2], Naama Keshet[2], Dinorah Barasch[1], Alina Nemirovski[1], Rakefet Czerninski[2]

[1]*Institute for Drug Research, The Hebrew University, Israel*

[2]*Faculty of Dental Medicine, Hebrew University - Hadassah School of Dental Medicine, Israel*

Squamous cell carcinoma (SCC) accounts for the majority of oral cancer cases and has poor prognosis. One of the significant alternations exhibited by oral SCC (OSCC) is the overactivity of the PI3K-AKT-mTOR (mammalian target of rapamycin) signaling, a central regulator of cell growth and proliferation.

Sirolimus was approved by the FDA in 1999 as an immunosuppressive agent. It is a macrocyclic lactone acting through mTOR pathway blockade. The antiproliferative effects of sirolimus and its analogues have been demonstrated on numerous cell types, explaining the development and use of these drugs in clinical practice. One of the studies has shown that the inhibition of mTOR by the chronic administration of sirolimus halts the malignant conversion of oral premalignant lesions and promotes the regression of advanced carcinogen-induced OSCCs.

In humans, there is no proven effective chemoprevention for the premalignant stage at the oral cavity; therefore, a new chemoprevention approach is needed. Local oral sustained release (SR) drug delivery systems (DDSs), coupled with drug targeting, enable to achieve a prolonged high drug concentration at the oral cavity by applying a significantly lower dose of the drug, thus enhancing its therapeutic potential while reducing its side effects and improving patients compliance.

In order to evaluate the local oral availability and the exposure of oral mucosa to the drug in transplant patients chronically-treated with sirolimus (PO). It has been shown that the saliva levels are significantly lower than blood levels.

We have further evaluated sirolimus saliva and blood levels following SR oral varnish DDS vs. mouthwash local application in healthy volunteers. We have found a 12 vs. 4 hours release at the oral cavity, respectively. Systemic exposure was limited, with a maximum level significantly lower than therapeutic, and safety was confirmed.

TARGETING OF LIPOSOMAL MITOMYCIN-C PRODRUG TO FOLATE RECEPTORS OF CANCER CELLS

Yogita Patil[1], Yasmine Amitay[3], Patricia Ohana[3], Hilary Shmeeda[2], Alberto Gabizon[1,2]
[1]*School of Medicine, Hebrew University, Israel*
[2]*Shaare Zedek Medical Center, Hebrew University, Israel*
[3]*Lipomedix Pharmaceuticals Ltd, Lipomedix Pharmaceuticals Ltd, Israel*

Mitomycin C (MMC) is a powerful anti-bacterial, antifungal and anti-tumor antibiotic often active against multidrug resistant cells. Despite a broad spectrum of antitumor activity, MMC clinical use is relatively limited due to its fast clearance and dose-limiting toxicity [1]. To exploit the potential antitumor activity of MMC and reduce its toxicity we have developed a lipophilic prodrug of MMC. A reducible prodrug conjugate of mitomycin-C (MLP) formulated in pegylated liposomes (PL-MLP), has displayed significant antitumor activity and reduced toxicity in mouse tumor models [2]. PL-MLP has minimal in vitro cytotoxicity unless reducing agents are added to the cell culture to activate the prodrug. In the present study, we hypothesized that targeting these liposomes via folate receptors will facilitate an intracellular activation of prodrug without added reducing agents. We formulated folate targeted PL-MLP liposomes with a folate conjugate and examined in vitro.

PL-MLP liposomes were prepared as described [2, 3]. MLP was incorporated with almost 100% efficiency into pegylated liposomes. For the cell uptake studies, ^{3}H-cholesterol-hexadecyl ether (^{3}H-CHE)-radiolabeled liposomes were prepared. The folate-conjugated PEG (5000)-DSPE ligand was post-inserted into preformed liposomes. This formulation was tested in vitro for cell uptake and cytotoxic activity in cell lines that over-express the folate receptor.

The presence of folate ligand did not interfere with prodrug activation in vitro. ^{3}H-CHE and MLP cell uptake levels were 4-fold and 9-fold greater in KB-FR cells when folate-targeted PL-MLP is compared to non-targeted PL-MLP. The cytotoxic activity of folate targeted PL-MLP liposomes was significantly increased up to 5-fold compared with PL-MLP liposomes in all tested folate receptor-expressing cell lines. Thus, folate targeting enhances liposome uptake by tumor cells enabling intracellular activation of prodrug in the absence of exogenous reducing agents, and leading to increased cytotoxicity in vitro. In vivo studies with folate-targeted PL-MLP are in progress.

TRENDING NOW: PATENTS FOR NANOPARTICLE BASED CARRIERS, RNAi MOLECULES AND OTHER MAJOR TOPICS IN DRUG DELIVERY

Joseph I. Wyse, Millie Kaplan Zeevi
Patent attorney firm, Dr. Eyal Bressler & Co., Israel

Drug delivery therapeutics are one of today's most rapidly advancing areas of medical research. The pharmaceutical field advances towards delivering drugs, bio-therapeutic molecules, nutrients or biosensors by specific organ/cell targeting, and controlled release administration, offering exciting promise for future therapies. New technologies such as novel compounds tailored for passing the physiological barriers in the human body, such as the skin, the gastrointestinal tract, the respiratory system, the eye, and the blood brain barrier, are constantly being developed. Recent advances in genetic engineering require the development of numerous new agents for delivery of nucleic acid molecules such as miRNA. Novel nanoparticle based carriers are being optimally designed to encompass multi components providing non-viral delivery solutions.

Are these new approaches being translated into commercial drug, diagnostic or theranostic activity which will benefit the patient? Are these new approaches able to leverage patent activity into an income stream for further research to offset R&D costs and make profits? The Patent field provides a wide scope of product development strategies. What are the patent 'hot spots' in the field of drug delivery? Inventions, encompassing the full scope of development, are all patentable assets. Are Israel's research efforts in this burgeoning field adequately served by patenting activity?

Our survey and analysis of the patent literature already provides some strong indications. The type of patent activity is presented, together with the patent activity of leading players, and strategies are suggested for Israeli researchers to maximize patent value and financial benefit of future advances in the field.

Methods for coordinating the R&D timeline, funding timeline, marketing timeline and regulation timeline with the patent lifecycle are presented. The patent implications of start –up –to – strategic partner joint ventures are addressed.

THE ROAD FROM IDEA TO PRODUCT VIA IP PROTECTION –THE INTRA CAVITARY DRUG DELIVERY DEVICE STORY

Joseph I. Wyse[1], Dan Sade Hochstadter[2], Millie Kaplan Zeevi[1]
[1]*Patent attorney firm, Dr. Eyal Bressler & Co., Israel*
[2]*Research and development, ICD, Israel*

Drug delivery and controlled release based consumer therapeutic products are one of today's most rapidly advancing areas of medical research, pharmaceutical business development, as well as non-pharmaceutical areas. Controlled release technology advances towards utilizing products for an increasing scope of fields ranging from treating disease to non-medical fields such as agriculture, cosmetics, consumer products, and environmental treatment. Many new approachesoffer exciting promise for future therapies.

The road from idea to product must be protected and assetized by tailored and precise intellectual property portfolio formation. Presented here is the story of the development of an intra cavitary drug delivery device, from the generation of an idea to the founding of a commercial company, ICD, accompanied by tailored IP protection.

The main ICD company product is an absorbable implantable drug delivery device that distributes the drug by infiltration of intra-peritoneal fluid through the device. Since the field of drug delivery is a highly patented field, rapid and focused patenting strategy was implemented. A highly sophisticated analysis of the prevailing patent landscape, and, crucially prediction of future patenting activities of the major entities and stakeholders. Following this, the company overall business strategy is matched with the current market status for such devices, the current technological deficit in such devices and the patentability of the company's invention.

PSORIASIS TOPICAL TREATMENT BASED ON MODIFEID STARCH AND ULTASOUND APPLICATION AS A DELIVERY SYSTEM FOR RNAi

Rinat Lifshiz[1], Galya Lerman[2,3], Riki Goldbart[1], Tamar Traitel[1], Dror Avni[2], Yechezkel Sidi[2,3], Joseph Kost[1]

[1]*Department of Chemical Engineering, Ben Gurion University of the Negev, Israel*

[2]*Center for Cancer Research and Department of Medicine, Laboratory of Molecular Cell Biology, Israel*

[3]*Sackler School of Medicine, Tel Aviv University, Israel*

Psoriasis is a chronic inflammatory skin disease that affects millions, and still without a cure. RNA interference (RNAi), including siRNA and miRNA, is a natural process of sequence-specific post transcriptional gene silencing to inhibit gene expression. We found few miRNAs which differs between normal and psoriatic involving skin[1], which can be an attractive therapeutic molecule for psoriasis treatment.

Two major barriers to topically delivered RNAi therapy exist: the barrier properties of epidermis's top layer (stratum-corneum), and naked RNAi stability *in-vivo* due to enzymatic degradation and immunological responses. Moreover, the efficiency of RNAi that does reach the target cells is further limited by poor cellular uptake.

To overcome these obstacles allowing topical delivery of RNAi to skin cells, one must consider the use of designed carrier for its efficient delivery to the desired target cells, combined with methods that enhance transdermal delivery. In this study, we suggested the use of ultrasound (US) as a mean to enhance biological membranes and skin permeability[2] and Quaternized starch (Q-starch) as an miRNA delivery carrier[3]. By combining these two approaches, the ability to bypass limiting barriers of skin permeability and keratinocytes cell transfection path is of high potential.

In-vitro experiments demonstrated the ability of Q-starch/RNAi complexes to enter human keratinocyte HaCaT cells. Moreover, US application allows introducing the cells siRNA concentration at two orders of magnitude lower then what was needed for transfection without ultrasound.

In-vivo experiments on SCID mice that were transplanted with human psoriatic skin verified the ability of US to enhance transdermal delivery for Q-starch/miRNA complexes to the keratinocytes cells at the basal layer of the psoriatic skin.

The use of US combined with Q-starch complexes has a potential to be an efficient tool that allows RNAi delivery across the stratum-corneum and allow uptake by keratinocytes for psoriasis treatment.

NOVEL MICROCAPSULES BASED PANCREATIC ECM FOR DIABETES THERAPY

Deborah Chaimov[1], Irit Meivar-Levy[2], Sarah Ferber[2], Marcelle Machluf[1]
[1]*Department of Biotechnology and Food Engineering, Technion, Israel*
[2]*The Endocrine Institute, Sheba Medical Ctr., Israel*

Microencapsulation of living cells in a biocompatible and semi-permeable polymeric membrane was proven to be an effective method for continuous drug delivery and for immunoprotection of the cells. Addition of extra cellular matrix (ECM) to the encapsulation system can regulate the activities of adherent cells, including proliferation and differentiation. The cellular secretion level of insulin can also be increased by activating the desired cell signaling pathway via integrin-ligand-bonds and subsequently stimulating the gene expression level. We propose to encapsulate human liver cells or human mesenchymal stem cells (hMSC) after transduction with Pancreatic and Duodenal homeobox 1(PDX-1), which induces trans-differentiation into functional insulin-producing cells, as a possible diabetic therapy application.

We designed and characterized novel engineered microcapsules containing 3D reconstructed pancreatic ECM. We have managed to decellularize and solubilize pancreatic matrix to form a nanofibrous gel that supports the adhesion, growth and biological function of cells. Moreover, we have shown that the gel is uniformly distributed throughout the entire microcapsule and reassembled at 37ºC into collagen fibers mimicking the natural ECM. We demonstrated that ECM-hMSC microcapsules offer immunogenic protection properties for the already hypoimmonogenic and pluripotent hMSC. In particular, the ECM–capsule provides a natural fibers niche that seems to significantly improve hMSC differentiation and insulin secretion. We have also found a higher insulin secretion from liver cells seeded on tissue culture plates coated with pancreatic ECM gels. Our *in vitro* and *in vivo* studies affirmed the lack of immunogenicity of our system. Currently we are working on *in vivo* feasibility studies.

Our findings demonstrate that the microenvironment within the microcapsule is permissive for cells survival and insulin secretion. Therefore, encapsulated insulin producing cells with native ECM can be considered as a platform to replace deficient pancreatic beta–cells while circumventing the shortage in tissue availability and the need for anti-rejection treatment.

MODIFIED STARCH BASED CARRIER AND ULTRASOUND APPLICATION FOR siRNA DELIVERY

Shachar Gat, Eliz Amar-Lewis, Riki Goldbart, Tamar Traitel, Joseph Kost
Chemical Engineering, Ben Gurion University of the Negev, Israel

RNA interference (RNAi) is a process of post-transcriptional gene regulation by which harmful genes can be “silenced” after delivering complementary short interfering RNA (siRNA) to target cells. The widespread use of RNAi therapeutics for disease's prevention and treatment requires safe and effective drug delivery vehicles since siRNA is highly degradable under physiological conditions. Therefore, modified cationic starch (Q-starch) was used as an siRNA carrier, due to its biodegradability, biocompatibility, low immunogenicity and minimal cytotoxicity.

Another challenge for RNAi pathway is passing the membrane of the target cells. Ultrasound (US) is an oscillating sound pressure wave. US pressures above a certain threshold can cause oscillating bubbles to collapse violently; a process known as inertial cavitation. Inertial cavitation is believed to temporarily improve the permeability of cell membranes, enabling the transport of extracellular molecules into viable cells.

The objective of this project is evaluating the ability of modified starch-based siRNA delivery system to induce gene silencing and assess the effect of ultrasound as a mean to enhance gene silencing.

In this work CT-26 cell line expressing green fluorescent protein was used as a cancer model. To investigate the effect of ultrasound on cells viability, CT-26 cells were exposed to low frequency US, after which cell viability was measured by MTT assay, and found that US intensity depends on the cells locations in the cell culture plate. Gene silencing efficiency was quantified by fluorescence activated cell sorter. Transfection experiment, using N/P=2 (ratio of protonated polymer amine groups to negatively charged nucleic acid phosphate groups) with and without exposure to US showed no significant silencing. Cellular uptake of the complexes was determined by image stream, the results showed that exposure to US did not enhance cellular uptake, therefore, cellular uptake is the main barrier for efficient transfection.

NANO-GHOSTS FOR DRUG TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER AS A POTENIAL THERAPY FOR NEUROLOGICAL DISORDERS

Noa Cohen, Marcelle Machluf
Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Israel

The central nervous system (CNS) is protected by the blood–brain barrier (BBB). The BBB, which is formed by impermeable tight junctions (TJs) between Brain Capillary Endothelial cells (BCEC), serves as an active and selective barrier regulating the homeostasis of the brain and protecting it from toxic substances. Unfortunately, the high selectivity of the BBB also hampers the passage of drugs. Therefore, despite the rapid development of drugs with well-established activity in the brain, many disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), brain tumors and Multiple Sclerosis (MS) remain severely undertreated.

In order to overcome these limitations, we aim to modify our stem cell nano-ghosts (NGs) drug delivery system to allow the transport through the BBB. The NGs are nano-vesicles produced from the plasma membrane of human mesenchymal stem cells (hMSCs) which possess membrane-associated targeting and migratory abilities to and through the BBB, and towards sites of inflammation. The NGs are expected to retain the cells' surface moieties and encompass their unique targeting capabilities, and therefore may serve as an effective drug delivery system for targeting neurological disorders.

In this work, we aim to study the transport ability of NGs across a 3D in-vitro model of the BBB, which is composed of a co-culture of rat glial cells and bovine Brain Capillary Endothelial Cells (BCEC). NGs will go under different modifications in order to improve their transport abilities, which will be tested for both a healthy and a diseased BBB.

ULTRASOUND APPLICATION FOR PSORIASIS TREATMENT BASED ON TOPICALLY DELIVERED miRNA

Hollander Etili, Rinat Lifshiz, Riki Goldbart, Tamar Traitel, Joseph Kost
Chemical Engineering, Ben-Gurion University of the Negev, Israel

Psoriasis is a chronic skin disease, caused by rapid and incomplete differentiation of skin basal cells. The treatments offered today, such as creams and steroids, provide only a short time relief. Micro interference RNA (miRNA) is a natural process of sequence-specific posttranscriptional gene silencing to inhibit gene expression. Studies have shown that certain proteins are expressed in psoriatic lesions more than in healthy skin, and the expression of a specific miRNA is reduced compared to the healthy subjects. Therefore, it could be an attractive therapeutic molecule for psoriasis treatment.

One of the main challenges for topically delivering miRNA to the basal cells is creating an effective path through the skin. Since skin is permeable to polar and charged molecules smaller than 500 Da, delivering miRNA (14 KDa) to skin basal cells needs to utilize methods, which enhances transdermal delivery. One of those methods is sonophoresis, in which application of ultrasound (US) is used to increase the permeability of skin by creating temporary tunnels for large molecules to penetrate into the skin, due to cavitation effect.

The aim of this research is to develop a noninvasive method for delivering miRNA molecules into the basal skin cells using ultrasound as a mean to enhance transdermal delivery.

Fluorescent microscope images of 5 microns thick porcine skin exposed to miRNA and treated with ultrasound, demonstrated that ultrasound exposure allowed the penetration of the miRNA molecule across the skin, compared to passive diffusion. A specific miRNA molecule penetrated all epidermal layers, even into the basal cell and into the nucleus.

MODIFIED STARCH BASED COMPLEXES AND ULTRASOUND APPLICATION TO OVERCOME siRNA TRANSPORT BARRIERS

Eliz Lewis[1,2], Riki Goldbart[1,2], Tamar Traitel[1,2], Dalit Landesman-Milo[3,4], Dan Peer[3,4], Joseph Kost[1,2]

[1]*Chemical engineering, Ben-Gurion University, Israel*

[2]*Ilsa Katz Institute for Nanoscale Science and Technology, Ben-Gurion University, Israel*

[3]*Department of Cell Research and Immunology, Tel Aviv University, Israel*

[4]*Materials Science and Engineering, Tel Aviv University, Israel*

RNA interference (RNAi) is a natural process of sequence-specific post-transcriptional gene silencing by which gene expression is inhibited and holds a promising future for therapeutic application[1]. The remaining challenge in RNAi before clinical use is developing an efficient and safe non-viral siRNA delivery mechanism. This study[2] focuses on non-viral delivery system based on modified starch (Q-starch) polysaccharide as siRNA carrier due to its natural characteristics such as biodegradability, biocompatibility, and minimal cytotoxicity. Another challenge in this therapeutic approach is overcoming the transport barriers along the delivery pathway. To address this issue, we suggest applying ultrasound in conjunction to the Q-starch delivery approach, since it has been shown to enhance drugs uptake into cells and tissues[3]. Therefore, the research objective is to establish and characterize Q-starch as siRNA carrier and investigate ultrasound effect on Q-starch/siRNA transport pathway into cells.

We demonstrated that Q-starch and siRNA are self assembled to form nano-sized complexes, which are stable upon degradation in human serum. In addition, gene silencing in the human ovarian adenocarcinoma cells, NCI-ADR/Res(NAR), resulted in efficient sequence specific gene knockdown after 72hr of incubation (~50% reduction). The effective reduction was time dependent, since after 24hr insignificant gene reduction was presented. The kinetic barrier was probably intracellular, since we efficiently established complexes' uptake during a 24hr course of study. However, application of ultrasound on NAR cells was able to overcome this barrier. We believe that ultrasound might have affected complexes' intracellular transport barriers since it insignificantly affected the uptake of the complexes into the cells.

In addition to the efficiency of the treatment, the safety was also evaluated and both approaches (ultrasound and Q-starch carrier) were found safe for NAR cells. Therefore, altogether the combination of ultrasound-assisted Q-starch/siRNA delivery mechanism provides effective RNAi therapeutic approach while maintaining the safety of the treatment.

HYALURONIC ACID BASED QUADRUGNOSTIC NANOPARTICLES FOR CANCER THERAPY

Ravit Edelman[1], Inna Levitzky[1], Tal Shahar[1], Anton Slavkin[1], Ruth Goldschmidt[1], Yehuda G. Assaraf[2], Yoav D. Livney[1]

[1]*Department of Biotechnology and Food Engineering, Technion-Israel Institute of Technology, Israel*

[2]*Department of Biology, Technion-Israel Institute of Technology, Israel*

Hyaluronic acid (HA) is a natural anionic polysaccharide and a chief component of the extracellular matrix in the body. It contributes significantly to cell proliferation and migration, hence is sought by certain cancers for tumor development and metastasis. Such tumor cells overexpress the CD44 receptor to uptake HA from the circulation.

Conventional chemotherapy requires high doses of drug combinations which cause severe toxic side effects and exhibits limited efficacy due to the frequent emergence of drug resistance, particularly multidrug resistance (MDR), e.g. efflux of a multitude of drugs by ATP driven transporters of the ABC superfamily, e.g. P-glycoprotein. An additional challenge posed by cancer is the diagnostic localization of tumors and their distant metastases.

In the current research we are developing a novel nanomedical platform which we term a "quadrugnostic nanoparticle"(QNPs), that is envisioned to be the next generation platform for simultaneous cancer diagnostics and therapeutics (theranostics): These QNPs harbor in the same vehicle, four synergistic components, including a selective targeting moiety, a cytotoxic drug, a chemosensitizer for overcoming a well-defined mechanism of MDR, and a diagnostic element for the localization of the malignant tumor and its distant metastases.The prototype QNPs are based on self-assembling conjugates of HA and a partly hydrophobic molecule (bovine serum albumin (BSA)). HA serves as the active-targeting moiety and as the targeting cancer cells overexpressing CD44 (e.g. ovarian cancer). Moreover, this QNP harbors a cytotoxic drug, a chemosensitizer that overcomes MDR by blocking a specific ABC drug efflux transporter and an imaging-aid. Results thus far reveal that the conjugates formed self-assembled NPs, bound hydrophobic cytotoxic drugs (Paclitaxel and C-1375 Imidazoacridinone), and were selectively taken up by cells overexpressing CD44 receptor but not by cells lacking CD44 and were more cytotoxic to those cells compared to free cytotoxic drugs because of active uptake by CD44.

TOXICITY OF FOLEY CATHETERS IS GOVERNED BY THEIR COMPOSITION AND STORAGE CONDITIONS

Mark Ryzov[1], Hen Popilsky[2], Pavel Gorenbein[2], **David Stepensky**[1]
[1]*Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel*
[2]*Drug and Medical Equipment Research & Quality Control Laboratory, The Medical Corps, Israel Defense Forces, Israel*

Introduction: Prolonged contact of Foley catheters with urinary epithelium cells leads to toxic effects, extent of which has not been fully elucidated. Extractable materials, which govern this toxicity, can be analyzed *in vitro* using L-929 mouse keratinocytes (the Pharmacopeial method) or T24 human urinary epithelium cells (the cells that are directly exposed to the Foley catheters *in vivo* and can be more suitable for toxicity assessment).

Objectives: To assess: 1) the *in vitro* toxicity of Foley catheters that differ in their composition (latex, silicon- or PTFE-coated latex, or latex-free); 2) the dependence of this toxicity on the duration and conditions (temperature and humidity) of storage.

Methods: We analyzed 24 batches of Foley cathetersthat were produced by different manufacturers and varied in their composition and storage time. Extracts of these catheters were incubated with L-929 and T24 cells, and changes in cell morphology and viability were analyzed using the light microscopy and the MTT test, respectively. Samples of Foley catheters were stored at elevated temperature and humidity conditions and their *in vitro* toxicity was analyzed using the same methods.

Results: Extracts of the majority of analyzed batches were highly toxic to both L-929 and T24 cells. The order of toxicity was: latex silicone-coated latex PTFE-coated latex, latex-free (silicone only) Foley catheters. There was no clear correlation between the extent of toxicity and the storage time. Elevated storage temperature decreased the toxicity and elevated humidity increased the toxicity of the analyzed samples.

Conclusions: Foley catheters release toxic extractables and induce toxic effects *in vitro*, and apparently also during their clinical use. Latex-free catheters are less toxic than latex-containing ones, and toxicity of a specific catheter depends on its storage conditions. We warrant against use of latex-containing Foley catheters in human subjects and recommend their replacement with latex-free catheters.

MECHANISMS OF CELL DEATH UPON EXPOSURE TO EXTRACTS OF DISPOSABLE MEDICAL DEVICES

David Stepensky, Luba Kozlovskaya
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel

Introduction: Extractables that are released from the individual parts of the infusion sets during their use induce local and systemic toxic effects, extent of which has not been fully elucidated. The safety of infusion sets can be analyzed *in vitro* using L-929 mouse keratinocytes (the Pharmacopoeial method) or brain endothelial cells (e.g., bEnd.3 or cEND cells, that originate from the cells that are directly exposed to the extractables from the infusion sets *in vivo*).

Objectives: To determine the relative toxicity of individual parts of infusion sets produced by different manufacturers. To reveal the mechanism of cell toxicity (i.e., apoptosis vs. necrosis) upon their exposure *in vitro* to the extracts of the individual parts of the infusion sets.

Methods: Toxicity of the individual parts of infusion sets was studied in bEnd.3 and L-929 cell lines. Changes in cell morphology and viability were determined using light microscopy and the MTT test, respectively. Detailed analysis of the mechanisms of cell death was performed using caspase test and membrane integrity test, Annexin V-FITC/7-AAD analysis by FACS, and DAPI staining followed by confocal microscopy.

Results: The toxicity of the individual parts was: latex tube cup. Detailed analysis of latex toxicity *in vitro* revealed impairment in membrane integrity, but lack of caspase 3/7 activities, indicative of cell necrosis. Exposure of the cells to the extracts of the tube and the cup induced accumulation of phophotidylserine on the cell membrane without any additional changes.

Conclusions: Extractables from the latex and the tube parts of infusion sets are toxic, apparently inducing cell necrosis. Extent of toxicity depends on the design, manufacturer, and storage time of the specific batch. Subsequent *ex vivo* and *in vivo* studies toxicity will help to reveal the extent of toxicity induced by the infusion sets during their use in the human subjects.

POSTER #52

ANTI-CANCER THERAPEUTIC POTENTIAL OF DORMANCY-ASSOCIATED MICRORNAS IN A HUMAN OSTEOSARCOMA DORMANCY MODEL

Galia Tiram[1], Ehud Segal[1], Rony Shreberk[1], Shiran Ferber[1], Paula Ofek[1], Taturo Udagawa[2], Liat Edry[3], Noam Shomron[3], Maayan Roniger[4], Batsheva Kerem[4], Yuval Shaked[5], Sarit Aviel-Ronen[6], Iris Barshack[6], Marcelo Calderon[7], Rainer Haag[7], Ronit Satchi-Fainaro[1]

[1]*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Israel*

[2]*Vertex, Vertex Pharmaceuticals, USA*

[3]*Cell & Developmental Biology, Sackler School of Medicine, Tel Aviv University, Israel*

[4]*Department of Genetics, The Life Sciences Institute, The Hebrew University, Israel*

[5]*Department of Molecular Pharmacology, Rappaport Faculty of Medicine, Technion, Israel*

[6]*Department of Pathology, Sheba Medical Center, Israel*

[7]*Institut für Chemie und Biochemie, Freie Universität Berlin, Germany*

It is well established that microRNAs (miRNAs) play a major role in tumorigenesis. However, their involvement in tumor dormancy has yet to be fully elucidated. We have developed a model of human osteosarcoma (OS) dormancy, which consists of a pair of cells generating either dormant avascular or fast-growing angiogenic tumors originating from the same parental cell line. Even though they exhibit similar proliferation and migration rate *in vitro*, these cells differ in their angiogenic activity and *in vivo* growth patterns. When inoculated into mice, Saos-2-E cells generate vascularized and palpable tumors within one month, while Saos-2-D cells remain avascular and non-palpable for a year. To explore miRNA regulation on the switch from dormancy to progressive disease, miRNA expression profiles of this pair of cells were compared. Three miRNAs were identified as potential regulators of OS dormancy: miR-34a, miR-93 and miR-200c. The expression pattern of these miRNAs was found to correlate with OS progression in clinical specimens. Analysis of formalin-fixed paraffin-embedded OS specimens showed that miR-34a, miR-93 and miR-200c are downregulated in both primary and metastatic tumors, compared to normal bones. The biological role of these miRNAs in tumor dormancy was validated via reintroduction into cells generating fast-growing tumors. Moreover, their therapeutic potential was determined using a polymeric nanocarrier. All dormancy-associated miRNAs evaluated were able to reduce angiogenic activity of fast-growing osteosarcomas and prolong their dormancy period. Taken together, these findings suggest that miR-34a, miR-93 and miR-200c have a role in OS dormancy and disease progression, partly by attenuating the angiogenic capabilities of this highly vascular malignancy, and can therefore be used as a dormancy-promoting therapy for OS.

DEVELOPMENT AND CHARACTERIZATION OF B-CASEIN BASED NANO-CARRIERS FOR ORAL DRUG DELIVERY

Tanya Turovsky[1], Irina Portnaya[2], Ellina Kesselman[2], Hadas Perlstein[3], Simcha Even-Chen[3], Yechezkel Barenholz[3], Abraham Rubinstein[3], Dganit Danino[1,2]

[1]*Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Israel*

[2]*Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Israel*

[3]*Laboratory of Membrane and Liposome Research, IMRIC, Hebrew University–Hadassah Medical School, Israel*

β-Casein is a 24 kDa milk protein classified as an intrinsically unstructured and amphiphilic polyelectrolyte. β-Casein possesses many properties facilitating its functionality in drug delivery systems, including excellent emulsifying and polymeric stabilizing abilities, and strong self-association tendency that leads to creation of nanometric core-shell micelles.[1]

We used these extraordinary properties to design a β-casein-based platform for oral delivery of poorly water-soluble drugs that suffer from low bioavailability, and developed simple, fast, reproducible, and efficient formulation.We found that β-casein can encapsulate variety of drugs in the form of stable micelles and nanoscopic complexes, showing the potential of the system to become a wide platform for oral delivery of insoluble drugs.

Here we discuss two widely-used NSAID drugs – celecoxib and ibuprofen. We show high drug loading for both, but structure that is drug-dependent. Extended work with celecoxib shows that the drug is solubilized in the hydrophobic micelle core in an amorphous form,[2] which facilitates drug penetration into the blood stream and results in increased drug bioavailability. Importantly, we achieved loading efficiency 96%, that allows to meet the high daily dose of this drug in 1gr of the dry dosage. Reversible reconstitution of the structures suggests possible drug administration also in liquid form. In-vivo experiments in big animals proved our protein micellar nanocarriers successfully stabilize, deliver, and effectively release celecoxib at the intestine wall.[3]

REFERENCES

1. Portnaya I. et al. Self-assembly of bovine β-casein below the isoelectric pH. J Agric Food Chem. 2008; 56(6):2192-2198.

2. Bachar M. et al. Development and characterization of a novel drug nanocarrier for oral delivery, based on self-assembled β-casein micelles. J Controlled Release. 2012; 160(2):164-171.

3. Perlstein H. et al. Beta-casein nanocarriers of celecoxib for improved oral bioavailability. Submitted.

LC100, A NOVEL PEGYLATED LIPOSOMAL DOXORUBICIN NANO-DRUG WITH GREATER SAFETY AND THERAPEUTIC EFFICACY THAN DOXIL®

Yaelle Bavli[1], Tal Berman[1], Ilan Winkler[2], Doron Friedman[2], Yechezkel Barenholz[1,2]
[1]*The Hebrew University - Hadassah Medical School, Laboratory for Membrane and Liposome research, IMRIC, Israel*
[2]*LipoCure Ltd, Jerusalem, Israel*

Doxil® (pegylated liposomal doxorubicin = PLD) is the first FDA approved (1995) nano-drug and is still in extensive clinical use. PLD is based on three principles: (i) prolonged drug circulation time and avoidance of the RES due to the use of pegylated nano-liposomes; (ii) high and stable remote loading of doxorubicin driven by a transmembrane ammonium sulfate gradient, which allows for drug release at the tumor site; and (iii) having the liposome lipid bilayer in a "liquid ordered" phase. Due to the EPR (enhanced permeability and retention) effect, Doxil is "passively targeted" to tumors. Doxil/Lipodox (approved generic) has a characteristic "coffee bean" shape due to its intraliposome long, stable crystals that impose on the nano-liposome a transformation from a sphere to a prolate ellipsoid shape (*Barenholz JCR 2012*).

Berman et al. abstract and poster describe the design, R&D and characterization of LC100; a spherical ~85 nm PLD in which the doxorubicin was remotely loaded by a transmembrane gradient of ammonium-methanesulfonate. The doxorubicin-methanesulfonate salt in the intraliposome aqueous phase doesn't form a long crystal and maintains the liposome in spherical shape. Moreover, the drug release rate of LC100 was significantly faster than of Doxil/Lipodox in tumor microenvironment conditions.

LC100 and Lipodox were compared in two studies: 1. Severity of palmar-plantar erythrodysesthesia (PPE) induced by liposomal doxorubicin products; 2. Evaluation of anti-tumor potential using a breast cancer xenograft model.

Results:

- The PPE model demonstrates the superiority (two tailed, t-test, p=0.0007) of LC100 over Lipodox in minimizing the severity of the dermal lesions.
- Tumor growth results revealed 69.3% and 55.2% inhibition for LC100 and Lipodox, respectively.

The results clearly show that the physical state of the intra-liposome drug affects nanodrug performance by decreasing toxicity while maintaining or even improving the therapeutic efficacy.

THE ANTI-INFLAMMATORY ACTIVITY OF A LEAD FUSED-CYCLOPENTENONE PHOSPHONATE COMPOUND AND ITS POTENTIAL IN THE LOCAL TREATMENT OF EXPERIMENTAL COLITIS

Dorit Moradov, Helena Shifrin, Efrat Harel, Mirela Nadler-Milbauer, Morris Srebnik, Abraham Rubinstein, Abraham Rubinstein
Faculty of Medicine, School of Pharmacy Institute for Drug Research, The Hebrew University of Jerusalem

Purpose. To screen a series of previously reported fused-cyclopentenone phosphonates in order to identify a lead compound and to explore its possible mode of action.

Methods. The ability of the compounds to reduce secreted TNFα levels by LPS-activated mouse peritoneal macrophages, as well as their cytotoxicity (MTT) in increasing concentrations was compared. A lead compound, namely, diethyl 3-nonyl-5-oxo-3,5,6,6a-tetrahydro-1*H*-cyclopenta [*c*]furan-4-ylphosphonate ("P-5") was identified. P-5 effect on IL-6, INFγ, MCP-1, IL-1α, MIP-1a and RANTES levels was tested *in vitro*, followed by an *in vivo* analysis in a colitis-induced rat model. Inflammation severity quantification was assessed macroscopically and by measuring tissue MPO and iNOS activity and TNFα and IL-1β levels. The levels of p38, IkBa and ERK and their phosphorylation products p-p38, p-ERK was compared by Western bolt.

Results. The longer the aliphatic side chains on the furan ring of the tested fused-cyclopentenone phosphonates, the better their anti-TNFα activity. P-5 reduced TNFα, IL-6, IL-1α, INFγ, MCP-1, MIP-1α and RANTES in LPS-activated macrophages. Moreover, it was effective in the local treatment of experimental colitis in the rat, where it inhibited mucosal MPO activity, reduced expression of iNOS and decreased mucosal levels of TNFα and IL-1β.

Conclusion. Although not having an inhibitory effect on human recombinant TACE, P-5 could be used for the spatial therapy of IBD (e.g. by colon-specific drug platforms). Its mode of action involves MAPKs through the ERK pathway but not through p38 and with no effect on IκBα, probably, with no effect on the expression of the NF-κB transcription factor.

POSTER #56

CHEMICAL MODIFICATIONS OF POLYSACCHARIDES: QUATERNIZATION OF VARIOUS POLYSACCHARIDES

Ramesh Chintakunta, Riki Goldbart, Tamar Traitel, Joseph Kost
Department of Chemical Engineering, Ben Gurion University of the Negev, Israel

In the last few years, considerable attention has been focused to the applications of natural plant polysaccharides due to their excellent properties. The cationic polysaccharides associated many advantages, they are water soluble, biocompatible, inexpensive, non-toxic and easily biodegradable. Due to these properties, they have been used in a various fields including textile, oilfield drilling, wastewater treatment and cosmetic industries.[1, 2] More recently, a large number of studies revealed that the natural plant polysaccharides and their derivatives have beenutilized in gene delivery, RNAi delivery and controlled release.[3]

Despite of their useful applications in various fields, the main disadvantage of native non cationic polysaccharides is insolubility in water and in most of the organic solvents. To overcome this disadvantage, insertion of cationic or anionic moiety onto the polysaccharides backbone is required.In this context, we added a cationic moiety onto the polysaccharides backbone and succeeded to improve their water solubility as well as mineral binding ability. The insertion of cationic moiety onto the polysaccharide backbone was achieved by the reaction of polysaccharide with 3-chloro-2-hydroxypropyltrimethyl ammoniumchloride (CHPTAC) in the presence of aqueous NaOH solution at room temperature. Using this procedure, we synthesized various quaternized polysaccharides such as Q-starch, Q-dextrin, Q-galactan, Q-pectin and Q-pectin-NH-Q.The obtained products were characterized by FT-IR, NMR, TGA and elemental analysis techniques. The application of quaternized polysaccharides to drug delivery and controlled release system is under progress in our research group.

REFERENCES

- Pal, S., Mal, D., & Singh, R. P. (2005). Cationic starch: An effective flocculating agent.Carbohydrate Polymers, 59, 417-423.
- Singh, R. P., Nayak, B. R., Biswal, D. R., Tripathy, T., &Banik, K. (2003).Biobasedpolymeric flocculants for industrial effluent treatment. Materials Research Innovations,7, 331–340.
- Fox, S. C., Li, B., Xu, D., & Edgar, K. J. (2011). Regioselective esterification and etherificationof cellulose: A review. Biomacromolecules, 12(6), 1956–1972.

POSTER #57

USE OF NEAR-INFRARED IMAGING FOR EVALUATION OF LIPOSOMES PHARMACOKINETICS IN A MURINE MODEL OF CEREBRAL MALARIA

Emma Portnoy[1,3], Natalia Vakruk[1,2], Ameer Bishara[1], Jacob Golenser[2,4], Shlomo Magdassi[3], Sara Eyal[1]

[1]*Institute for Drug Research, Hebrew university, Israel*

[2]*Department of Microbiology and Molecular Genetics, Hebrew university, Israel*

[3]*Casali Institute, Institute of Chemistry and Center for Nanoscience and Nanotechnology, Hebrew university, Israel*

[4]*Department of Pathology and Bosch Institute, University of Sydney, Austria*

Cerebral malaria (CM) is the most severe neurological manifestation of malaria. Attempts have been done to encapsulate antimalarial drugs in liposomes for the treatment of CM. However, the pharmacokinetics of liposomes in CM has been explored only scarcely.

Indocyanine Green (ICG) was attached to PEGylated (PEG-LP-ICG) or non-PEGylated (LP-ICG) liposomes by passive adsorption. Liposomes were injected intraperitonealy or intravenously to naïve or *Plasmodium Berghei Anka*-infected C57 Black mice. Mice were imaged *in-vivo* or ex-vivo, 4 hr post injection. Mice received IP with 5mg/kg of arthemison or vehicle starting from day 3 post infection for 3 days, studies were conducted on day 6 post infection.

LP-ICG exhibited significant accumulation in brains of PbA infected mice compared to control (p0.01) on days 6 and 7 post infection. PEGylation significantly (P0.05) increased the blood emission intensity of liposomal ICG in both naïve and infected mice (2.5- and 1.6-fold, respectively). However, brain emission intensity was increased by PEGylation in naïve mice only (2.1-fold; P0.01). LP-ICG exhibited significant accumulation (4.2-fold; p0.01) in brains of PbA-infected, non arthemison-treated mice on day 6 post infection.

Near infra-red imaging can be used to study liposomes pharmacokinetics in murine malaria models. As in healthy mice, PEGylation increases liposomes blood concentrations in mice with CM. However, PEGylation does not increase their brain accumulation in infected mice. These altered liposomes pharmacokinetics may have implications on drug delivery, effectiveness and toxicity in the treatment of CM.

ORAL DELIVERY OF POLYMERIC NANOMICELLS AS A PLATFORM FOR IMPROVING BIOVAILABILITY OF POORLY SOLUBLE DRUGS

Eva Abramov, Mara Shapero, Ofra Benny
The School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Poorly soluble drugs present a significant challenge in oral drug delivery development as the gastrointestinal track, with its wide range of pH and enzymatic activity, acts as a significant physiological barrier.

Our previous studies demonstrated that the conjugation of lipophilic drugs to the short polymer monomethoxy poly lactic acid polyethylene glycol (mPEG-PLA) improved drug solubility, stability and oral availability. In spite of the promising results, the use of chemical conjugation is limited by the availability of appropriate functional groups in a drug, and, in many cases, the drug loses activity upon conjugation. Therefore, we are now focused on developing a platform oral drug delivery system based on encapsulation without conjugation.

The mPEG-PLA di-block copolymer is known to form ~20nm nanomicelles through self-assembly in aqueous solution. To investigate the intestinal absorption of such solid nanomicelles, we employed the cellular model of caco-2 permeability assay. In order to elucidate the molecular mechanism of mPEG-PLA nanomicelle endocytosis in cells, specific inhibitors of clathrin, caveolae and lipid raft mediated endocytosis were used.

The results showed fluorescent labeled mPEG-PLA nanomicelles in caco-2 cells as early as 15 min post incubation and kinetics studies showed that the apical to basolateral apparent permeability coefficient (Papp) was 3.8 x10-6 cm/s after 2 hours and 5x10-6 cm/s after 4 hours, indicating good intestinal absorption. The mPEG-PLA nanomicelles penetration potential across the caco-2 monolayer supports their use as a platform technology for the oral delivery of poorly absorbed drugs. Our findings broaden the possibilities of oral drug delivery and carry significant clinical relevance, particularly for long-term therapy of chronic diseases.

AMPHIPHILIC PEG-DENDRON HYBRIDS AS A MODULAR PLATFORM FOR ENZYME-RESPONSIVE NANOCARRIERS

Ido Rosenbaum, Assaf J. Harnoy, Marina Buzhor, Roey J. Amir
School of Chemistry, Tel Aviv University

Enzyme-responsive micelles have great potential as drug delivery platforms due to the high selectivity of the activating enzymes. We have developed a modular design for the efficient and simple synthesis of amphiphilic block copolymers composed of linear hydrophilic polyethyleneglycol (PEG) block and an enzyme-responsive dendron as the hydrophobic block. The amphiphilic nature of these hybrids results in their self-assembly in water into micellar nanocarriers, which can disassemble and release encapsulated or covalently attached molecular cargo upon enzymatic activation. Such smart amphiphilic hybrids could potentially be use as nanocarriers with adjustable release rates for biomedical delivery applications.

POSTER #60

TARGETED THERAPY OF METASTATIC CANCER USING CELLS DERIVED NANO-GHOST

Natali Malkah, **Stasia Krishtul**, Naama Toledano Furman, Marcelle Machluf
Faculty of Biotechnology and Food Engineering, Technion- Israel Institute of Technology, Israel

One major challenge in cancer therapy concerns the formation of metastasis, the spread of cancer cells from the initial tumor to other parts of the body. Despite the improvement in cancer treatment, many drugs fail to reach sites of metastases.

We propose to utilize Nano-Ghosts (NGs), our novel drug delivery system, to selectively target metastasis while delivering therapeutic drugs. NGs are vesicles produced from the plasma membrane of human mesenchymal stem cells (hMSCs), known for their natural targeting of multiple cancers and hypo-immunogenicity. As a model, we chose to target metastatic non-small cell lung carcinoma (NSCLC), the most common lung malignancy, which holds the highest mortality rate of all cancers. Two platinum based anticancer drugs were evaluated– cisplatin and carboplatin, the first being the drug of choice for treating NSCLC. Platinum based anticancer drugs are widely used in the clinic, treating a variety of tumors, however their clinical use has been impeded by their sever cytotoxicity, leading to agonizing side effects. Nanocarrier-based delivery is one approach trying to address this issue, in order to improve current platinum chemotherapy.

We were able to design a NGs system encapsulating these two drugs, while using two drug encapsulation methods: extrusion and electroporation. TEM imaging assured the formation of the Pt-NG and dynamic light scattering analysis confirmed that the NGs average diameter size was 260 nm and average zeta potential was -17 mV. Drug: lipid ratio and encapsulation efficacy rates of cisplatin were determined using ICP, and found to be highest using electroporation (0.15 µg/µg and 6.6%, respectively). Cisplatin showed higher stability when dissolved in saline as compared to DMSO. Interestingly, the results show that incorporation of platinum drugs in NGs, improved their therapeutic index toward NSCLC cell line A549 compared to the free drug (30% differences in cell viability).

THE SHELF-LIFE STABILITY AND PROSPECTS FOR USING VESICLES DERIVED FROM THE CYTOPLASMIC MEMBRANE OF MESENCHYMAL STEM CELLS FOR CANCER IMAGING

Miguel Fliman, Marcelle Machluff
Biotechnology and Food Engineering, Technion, Israel

Recently we reported on the development of a novel drug-delivery system based on vesicles composed from the cytoplasmatic membranes of mesenchymal stem-cells (MSC), known for their natural targeting of multiple cancers. Encompassing MSC surface proteins and armed with their unique targeting capabilities, these vesicles (also termed NanoGhosts or NGs) may be loaded with various drugs and selectively targeted against various cancers at different developmental stages. Accordingly, we believe that such NGs can be also utilized for the delivery of contrast agents and therefore used for imaging of otherwise non-detectable or sub-metastatic tumors. A procedure was developed to load the NGs with 20-nm particles of Maghemite (a widely used MRI contrast agent) and retrieving them without centrifugation; which would be useless due to the similar densities of the loaded NGs and the free Maghemite. The NGs shelf-life stability in PBS (with or without 10% DMSO) was assessed at different temperatures (4^{o}C, -20^{o}C and -152^{o}C) for up to several weeks by following the NGs size (DLS), charge (Zeta potential) and morphology (Cryo-TEM). At room temperature the NGs aggregated in several hours, however remained stable at 4^{o}C for over 10 days which is suitable for their short-term storage. In long-term storage, the NGs were found most stable in PBS without DMSO at -20^{o}C, maintaining the same charge and size for more than two months. However, the NGs morphology was best preserved when rapidly frozen in liquid nitrogen (-152^{o}C) prior to storing them at -20^{o}C. These results collectively indicate that the NGs can be loaded with and potentially used for the delivery of MRI contrast agents. The NGs' stability, which enables their long-term preservation, adds to the technological value of this application that we expect to have a significant impact on cancer imaging and diagnostics.

THERMODYNAMICS REVEALS SURPRISING FEATURES OF THE FIRST FDA-APPROVED NANODRUG, DOXIL (OR ITS GENERIC, LIPODOX)

Xiaohui Wei[1,2], Rivka Cohen[1], Chezy Barenholz[1]
[1]*Biochemistry, Laboratory of Membrane and Liposome Research, The Hebrew University-Hadassah Medical School, IMRIC, Israel*
[2]*Pharmaceutics, School of Pharmacy,Shanghai Jiao Tong University, China*

The thermodynamics of Doxil® (Caelyx®) the first FDA-approved (1995) liposomal drug and Lipodox®, its first FDA-approved (2013) generic, has not been described before. Our DSC studies of this nano-drug reveal surprising features of the nano-liposome membrane and its intraliposomal doxorubicin-sulfate nanocrystal. The Lipodox membrane shows a small reversible endotherm upon heating (at 53°C), despite containing high percentage of cholesterol (35 mole%).The presence of DSPE-PEG2k seems to increase the cooperativity of this lipid phase transition. Passing through this phase transition does not result in doxorubicin leakage. The intraliposomal doxorubicin-sulfate nanocrystals melt at ~70°C, much higher than the ~63°C melting of the dox-sulfate crystals in the 250mM ammonium sulfate bulk phase, suggesting an effect of the nanovolume. For both crystals (the nano and the bulk), the crystallization and melting are reversible. Our study demonstrates the power of DSC in nanodrug characterization.

AEROSOL SYNTHESIS OF THERMOLABILE NANOPARTICLES WITH CONTROLLED PROPERTIES

Amol Pawar, Chandra Venkataraman
Chemical Engineering, Indian Institute of Technology Bombay, India

Therapeutic nanoparticles with controlled properties such as size and structure enable control over drug bioavailability and therapeutic outcomes. This work addresses the challenge of processing thermolabile biomaterials and biomolecules into nanoparticles without compromising structural integrity and activity.

Design of a pulse-heat aerosol reactor that enables control over time–temperature history and resulting evaporation rates/drop temperatures, to achieve control over particle properties was demonstrated. Control of particle size (50-200 nm), structure (solid and shell-type) and crystallinity (20-80%) of stearic acid nanoparticle aerosol lipid matrices (NALM) was achieved through manipulation of precursor properties (solvent vapour pressure and solute concentration) and heat–pulse levels. Links between particle properties and processing conditions were elucidated using a combination of experimental and computational studies. The formation of shell–type rather than solid nanoparticles, at higher evaporation rates, was in good agreement with the model predictions, related to the development of steeper solute gradients within the drop at such evaporation rates. A correlation between measured crystallinity and simulated droplet evaporation rate/drop temperature, implying influence of temperature on nucleation/ordering processes during formation of solid phases within the droplet was established. Activity of glucose oxidase nanoparticles was retained when subjected to high pulse-heat processing (gas temperature of 110 °C). Model predicted drop temperature, under high pulse heating, was significantly lower than gas temperature from evaporative cooling, and below temperatures of melting of stearic acid and denaturation of glucose oxidase.

Nanoparticles of the sizes and structure obtained in the pulse-heat aerosol reactor (PHAR) are suitable for several applications including tumor or brain targeting, enhanced cellular uptake and controlled drug release. Through this work, pulse–heat aerosol processing is established as a single–step, continuous method to process heat–sensitive biomaterials and biomolecules into nanoparticles with controlled properties, while avoiding thermal damage.

NANOPARTICLES FOR THE DELIVERY OF PIPTIDES TO THE BRAIN

Moran Haim Zada[1], Wahid Khan[1], Michael J. Kubek[2], Abraham J. Domb[1]
[1]*Institute of Drug Research, School of Pharmacy-Faculty of Medicine, The Hebrew University of Jerusalem, Israel*
[2]*Departments of Anatomy and Neurobiology, Anatomy & Cell Biology and Psychiatry, Indiana University School of Medicine, USA*

Delivery of peptidic drugs in the brain is a significant challenge, unless the drug in injected to the brain. Peptide drugs are commonly delivered by IV or SC injections and do not cross the BBB unless there is an active carrier that crosses the BBB and delivers the drug within the brain [1,2].

The objective of this study was to develop a robust process for the manufacturing of TRH loaded nanoparticle in poly(sebacic anhydride), a fast degrading polymer. These nanoparticles are used to deliver peptide drugs directly to the brain neurons via nasal spray through the olfactory site.

Thyrotropin-releasing hormone (TRH, protirelin), a brain-derived neuropeptide, have a rapid onset efficacy against suicidal ideation and severe depression.

The preparation of nanoparticles was carried out by solvent/anti solvent precipitation method. Nanoparticles of a particle size of 200-500 nm and the zeta potential was -50 mV with high TRH loading was obtained. TRH was constantly released for 12 hours while P(SA) being hydrolyzed. These nanoparticles are being considered for clinical trials to reduce the number of suicides in the US army.

REFERENCES

[1] Kubek MJ, Kubek DJ, Domb AJ, Veronesi MC. Thyrotropin-releasing hormone (TRH): Intranasal delivery to seizure foci. Epilepsia 2007;48:310-311.

[2] Kubek MJ, Domb AJ, Veronesi MC, Attenuation of Kindled Seizures by Intranasal Delivery of Neuropeptide-Loaded Nanoparticles, Neurotherapeu.6(2), 359-371, 2009.

DEVELOPMENT OF ELECTROPHILE – DELIVERY - SYSTEMS (EDS); TARGETING THE NRF$_2$-KEAP1 PATHWAY IN SKIN BY UNIQUE ELECTROPHILES

Maya Ben Yehuda Greenwald[1,2,3], Shmuel Ben Sasson[3], Havazelet Bianco- Peled[2], Ron Kohen[1]

[1]*The Institute for Drug Research, School of Pharmacy, Hebrew University of Jerusalem, Israel*

[2]*Department of Chemical Engineering, Technion-Israel Institute of Technology, Israel*

[3]*4Department of Developmental Biology and Cancer Research, Hebrew University of Jerusalem, Israel*

The nuclear factor erythroid 2–related factor 2 (Nrf2) is an emerging regulator of cellular resistance and protecting enzymes such as the phase II enzymes. Activation of the Nrf$_2$-keap1 pathway may have a beneficial effect in the treatment of a large number of skin disorders including inflammatory diseases, irradiation damage and some types of cutaneous malignancies, by stimulating the endogenous defense mechanism. However, prolonged and enhanced activation of this pathway is detrimental and limits the therapeutic potential of Nrf$_2$-keap1 activators. Therefore, controlled and transient activation of this pathway presents a novel strategy for skin protection/treatment under various stress conditions and different skin pathologies.

Designing a unique electrophile delivery system which would target the Nrf2-Keap1 pathway and control therapeutic effect presents a promising approach. Moreover electrophile delivery system will ensure the delivery of the electrophiles into skin cell's cytoplasm.

Electrophiles were encapsulated into a nanotechnologically based dermal delivery system composed of biocompatible excipients, enabling Nrf$_2$ activation and induction of phase II enzymes. The nanometric size and shape of the dermal drug delivery system displayed high similarity, indicating high stability. Our studies demonstrated that EDS were more potent inducers of Nrf2-keap1-ARE pathway than the free electrophiles, causing the activation of phase II enzymes and providing skin protection

The activity of new unique electrophiles and their EDS will be described

E-SELECTIN TARGETED COPOLYMER CONJUGATE FOR TARGETING INFLAMED TISSUES

Aviad Avda[1], Dalia Tyomkin[1], Jonathan Lior[2], Ayelet David[1]
[1]*Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel*
[2]*Neufeld Cardiac Research Institute, Tel Aviv University, Sheba Medical Center, Tel-Hashomer, Israel*

Actively targeted polymer conjugates show promise in increasing drug concentrations at the affected areas while decreasing accumulation in near non-target organs. E-selectin is a cell adhesion molecule expressed exclusively on endothelial cells activated by pro-inflammatory cytokines. It plays an important role in cell binding and recruitment both in inflammatory diseases and metastatic dissemination. Our group has recently demonstrated that FITC-labeled HPMA copolymer conjugated to the high affinity E-selectin binding peptide (ESBP, primary sequence DITWDQLWDLMK) as well as to Doxorubicin (DOX) (via pH-sensitive linkage), actively target tumor vascular endothelial cells, via E-selectin mediated interaction[1]. This copolymer also demonstrated a 150-fold improved cytotoxicity relative to non-targeted P-DOX conjugates, towards human vascular endothelial cells. We hypothesized that HPMA copolymer conjugated to ESBP can also serve as a drug targeting platform for inflammatory diseases. The FITC-labeled HPMA copolymer precursor having active ester groups for peptide attachment and hydrazone groups for drug conjugation (designated as P-(GG-ONp)-(GG-HzBOC)-FITC, where P represents the HPMA copolymer backbone) were synthesized by random radical precipitation copolymerization. Esbp peptide with N-term lysine and arginine substituted for lysine (primary sequence: KDITWDQLWDLMR, designated as Esbp(KR)) was coupled to the precursor copolymer (P–(GG–ONp)–(GG-HzBoc)-FITC) via ONp aminolysis. The anti-inflammatory drug Dexamethasone (DEX) was attached to the polymer via the pH sensitive hydrazone bond. The ability of P-(Esbp)–(GG-HzBoc)-FITC to target vascular endothelium in inflamed tissues in hind-limb ischemia and inflamed endothelium in atherosclerosis model was tested. The *in vivo* staining of the endothelial lining of blood vessels was greater in P-(Esbp)-(GG-HzBoc)-FITC treated group when compared to the control, non-targeted P-(GG-OH)-FITC, in the different models tested. HPMA copolymer-Esbp conjugate bearing Dexamethasone (P-(Esbp)-(DEX)-FITC) is currently being investigated for its therapeutic effects *in vitro* and *in vivo*.

TARGETING METASTASIS WITH NANOMEDICINE

Dikla Vardi-Oknin, Evgeniya Goldman, Janna Shainsky, Avi Schroeder
nanotechnology, Technion, Israel

A great therapeutic challenge lies in the treatment of metastatic cancer. For example, although early detection of breast cancer increases survival, the 5-year survival rate of patients diagnosed with metastatic breast cancer remains below 15%.

Nanotherapeutics offer many potential benefits such as reducing the side-effects of chemotherapeutic agents, protecting the encapsulated drug from degradation, carrying multiple drugs concomitantly and targeting specific tissues. To date, nanotherapeutics have taken advantage of the enhanced permeability and retention effect (EPR) to target well-vascularized primary tumors. Contrarily, small metastases are usually poorly-vascularized, thereby thought to hinder the use of nanoparticles.

Here, we studied the biodistribution of 100-nm PEGylated liposomes to metastatic sites. Using two *in vivo* screening methods (whole animal fluorescent imaging and inductively coupled plasma (ICP) spectroscopy), we detected the liposomes in triple-negative breast cancer (4T1) metastasis, embedded experimentally in the lungs of BULB/c mice.

The liposomes peaked in the metastasis 12 hours post intravenous injection. Using a combined model, of animals bearing both a primary tumor and lung metastasis, we noticed that the primary tumor acts as a sink, decreasing the liposomal accumulation in the metastatic tissue. Interestingly, the accumulation of liposomes in the metastasis, as a function of the metastatic progression, displayed a step-wise targeting profile. For the first ~10 days post metastatic induction no liposomes were detected in the lungs; later, a significant step-wise increase in nanoparticle accumulation in the metastasis was detected. Since metastases were not observed at this time, the phenomenon seems to be due to the formation of a pre-metastatic niche in the lungs.

This study grants insights into the profile of nanoparticle distribution to metastatic sites and may have future clinical implications for the use of nanomedicines for treating metastatic cancer.

TOWARDS BODIPY-CORED NEAR IR-EMITTING WATER-SOLUBLE DENDRITIC PLATFORMS

Ramesh Palakuri, Jeny Kuks, Moshe Portnoy
School of Chemistry, Tel-Aviv University, Israel

Water-soluble dipyrromethene boron difluoride (BODIPY) derivatives were synthesized for biological imaging applications. The series of novel water-soluble BODIPY dyes have been obtained by functionalization of BODIPY moiety with oligo(ethylene glycol)-decorated dendrons.

BODIPY dyes with extended π-conjugation systems carrying long alkyl chain-decorated dendrons were also prepared. These structures are typically insoluble in water, but are soluble and highly fluorescent in common organic solvents, such as toluene, dioxane and isopropanol.

In order to demonstrate feasibility of using branched oligo(ethylene glycol)-carrying building blocks to promote water-solubility of BODIPY dyes with extended π-conjugation system, we prepared BODIPY dyes bearing monostyryl and distyryl groups at positions 3,5 (**G2P1** and **G2P2**, respectively) by condensing the methyl substituents of the BODIPY core at positions 3,5 with dendritic salicylaldehyde derivatives bearing omega-Me-tris(ethylene glycol) terminal groups.

Both BODIPY dyes **G2P1** and **G2P2** exhibit a very good fluorescence quantum yield of 69% and 16%, respectively, in ethanolic solution. We can increase the water solubility of such BODIPY dyes by using derivatives based on extended oligo(ethylene glycol) chains or glycerol-derived diol terminal groups.These BODIPY dyes are highly water soluble because of the strong hydrophilic nature of oligo(ethylene glycol)methyl ethers and glycerol residues. This approach offer highly efficient ways to prepare different BODIPY dyes with emission ranging from green to near IR regions. All BODIPY dyes are easily soluble not only in water but also in common organic solvents, such as ethanol, dioxane, acetonitrile.

These types of BODIPY-based dendritic platforms, particularly the near IR emitting derivatives, could be readily applied in medicinal chemistry. For instance, we can functionalize the dendritic building block phenols with propargyl connector groups, prior to condensation with the BODIPY core, for subsequent attachment of payload.

KEYWORDS

Fluorescence, dendrimers, near IR emission.

TARGETING CD44 OVEREXPRESSING PROSTATE CANCER CELLS USING LAMININ DERIVED PEPTIDE POLYMER CONJUGATE

Michal Zaiden, Ayelet David
Department of Clinical Biochemistry and Pharmacology, Faculty for Health Sciences, Ben Gurion University in the Negev, Israel

The usage of current anti-cancer drugs is limited due to their low solubility and nonspecific toxicity. Among the variety of polymeric drug carriers, N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer is an excellent anticancer drug carrier owing to its good water solubility, enhanced tumor accumulation, reduced non-specific toxicity, low immunogenicity and easy for conjugation. CD44 is overexpressed in many cancer cells, among them prostate tumor cells. It is known for its ability to contribute to both refractory and metastatic prostate cancer. A5G27 laminin-derived peptide (K-RLVSYNGIIFFLR), a ligand of CD44, blocks FGF2 activity by binding to heparin sulfate side chain. In this work we aimed to design and develop novel CD44-targeted HPMA copolymer drug conjugates containing A5G27 that can recognize cancer cells that overexpress CD44 receptor as well as inhibit tumor sphere formation, cell migration, invasion and metastasis. The use of the HPMA-based docetaxel (DTX) conjugate P-(A5G27)-DTX, may improve the outcome of prostate cancer treatment due to its ability to target and eliminate CD44(+) prostate cancer cells. HPMA-based precursor polymer carrying fluorescein-isothiocyanate (FITC) and active ester (p-nitrophenyl, ONp) groups (designated as P-(GG-ONp)-FITC) was synthesized by random radical precipitation copolymerization and characterized by FPLC and spectrophotometry. The copolymer was further conjugated to A5G27 as a targeting moiety and potential therapeutic agent via ONp aminolysis, and characterized using ^{1}H-NMR. Our primary in-vitro result shows good correlation between CD44 levels and the binding and internalization of P-(A5G27)-FITC to PC3, MSC and LNCaP cells, when compared to the non-targeted control copolymers P-(GG-OH)-FITC or P-(Scrm)-FITC, as determined by FACS analysis. Confocal fluorescence images confirmed the selective internalization of P-(A5G27)-FITC and localization at endosomal and lysosomal compartments. These results indicate the potential use of A5G27 as a targeting moiety towards prostate cancer cells that over-express CD44.

IN-VITRO AND IN-VIVO EVALUATION OF OPA, A POTENT OXALIPLATIN DERIVATIVE

Aiman Abu-Ammar, Taher Nassar, Dan Gibson, Simon Benita
The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Platinum (Pt)-based anticancer drugs are widely used for treatment of a variety of solid tumors. The therapeutic outcome of Pt drugs is limited not only by serious side effects, but also by intrinsic or acquired resistance of cancer cells. Oxaliplatin was the first drug approved to be able to overcome cisplatin resistance.

In this study a new lipophilic derivatives of oxaliplatin were synthesized for the purposes of improving drug performance and enhancing cytotoxicity in sensitive and resistant cancer cell lines with minor side effects. Furthermore, the potential therapeutic advantage of incorporating such compounds in nanoparticles (NPs) as a delivery system was evaluated.

OPA was selected as the lead compound of these new compounds, chemically identified using ^{1}H-NMR, ^{195}Pt-NMR, HPLC and elemental analysis. Its lipophilic character and experimental LogP was evaluated. OPA NPs were prepared and characterized by TEM, SEM and AFM. Their mean diameter ranged between 150 and 230 nm. High encapsulation yields of OPA were obtained (95%), with a drug content in NPs varying from 21.5 to 22.7% w/w. OPA NPs were successfully lyophilized and remained stable over 16 weeks at -20°C. Free oxaliplatin, OPA and OPA NPs were incubated over 120h with SKOV-3 and SKOV-3-luc cell lines to assess cytotoxicity. OPA and OPA-NPs exhibited pronounced cytotoxic effect compared to oxaliplatin, due to increased cellular uptake and DNA platination. OPA and OPA-NPs exhibited a greater tumor growth inhibition than oxalipaltin and respective controls in an orthotopic intraperitoneal model of metastatic ovarian cancer in SCID-bg mice. OPA; significantly reduced the average bioluminescence at weeks 9 and 10 following tumor inoculation.

Taken together, potent Pt(IV) derivative of oxaliplatin was developed and characterized *in-vitro* and *in-vivo*. The preliminary results showed high potency of OPA and OPA-NPs over the native molecule. These encouraging results need to be further studied and confirmed.

DRUG RELEASE OF LIPOSOMAL FORMULATIONS: THE BASIS FOR IN-VITRO – IN-VIVO CORRELATION IN LIPOSOMAL DRUG DEVELOPMENT

Genia Levinton Shamuilov[1], Ilan Winkler[1], Katia Ioffe[1], Yaelle Bavli[2], Nofar Keren[1], Doron Friedman[1,2], Dima Shamrakov[1], Chezy Barenholz[2]

[1]*Lipocure, Jerusalem Biotechnology Park, HadassaheLtd, Israel*

[2]*Laboratory of Liposome and Membrane Research, IMRIC,, Hebrew University-Hadassah Medical School, Israel*

FDA defines "In-Vitro In-Vivo Correlation" (IVIVC) as a "predictive mathematical model describing the relationship between an in-vitro property of a dosage form and an in-vivo response". Generally, the in-vitro property is the rate or extent of drug dissolution or release, while the in-vivo response is the plasma drug concentration.

The meaningful and productive use of IVIVC in the pharmaceutical development requires in-depth understanding of composition/structure/function relationships. This knowledge also allows development of a reproducible manufacturing process of the drug product, which is another major requirement of NDA and ANDA approval by the FDA.

Establishing IVIVC of liposomal drugs and nano-drugs is challenging due to the product complexity, originating from the fact that liposomes are supramolecular assemblies.

We designed two in-vitro drug release methodologies. One for evaluating nano-liposomes for systemic injection and the second, for large liposomes / hydrogel "two-stage system" for local injection.

In-vitro release of intravenously injected nano-liposomes is tested using compendial instrumentation at physiologically relevant conditions. Strong ion-exchange resin is used to bind the free drug, thus providing "sink" conditions. The concentration of encapsulated drug in the solution is monitored spectrophotometrically.

Release of large liposomes in locally injected hydrogel is tested on the same equipment. The concentration of released drug is monitored by HPLC with UV detection. As the hydrogel is administered as a local subcutaneous prolonged-release depot, an enhancer cell fitted with an appropriate membrane is used to simulate the release and absorption in tissues.

Slow release profiles are agreeable with pharmacokinetic behavior.

FTIR AS AN IN-PROCESS CONTROL METHOD TO EVALUATE LIPIDS CONTENT

Alexander Lyskin[1], Doron Friedman[1], Michael Raslin[1], Yechezkel Barenholz[2], Dima Shamrakov[1]

[1]*LipoCure Ltd, Biotechnology Park Hadassah, Jerusalem, Israel*

[2]*The Hebrew University-Hadassah Medical School, Laboratory of Membrane and Liposome Research, Jerusalem, Israel*

LipoCure is developing a number of highly-potent therapeutics utilizing proprietary liposome-based nano-drugs with novel drug-loading methods and drug-release mechanisms. In the course of liposome-based nano-drugs manufacturing, in-process determination of total lipids content is a critical step during production.

Currently this analysis is performed by a lengthy HPLC procedure, which interrupts the production for many hours while waiting for the HPLC analysis results.

Therefore a rapid alternative procedure was introduced, where the "in-process" samples are assayed "as is" using an FTIR spectrophotometer equipped with multiple bounce Zn-Se ATR (attenuated total reflectance) sampling module. The measurement is based on asymmetric stretch vibrations of the phosphate band at ~1232cm^{-1}.

In this work, we developed and validated a new rapid method to evaluate lipids content in-process by FTIR. The FTIR method is based on generation of a calibration curve with standard solutions. In the validation work, we found good linearity, accuracy and precision of the method. In addition, we verified this method by the HPLC/Evaporative Light Scattering Detection (ELSD) validated method. Therefore, measurements by the FTIR method can be performed for the lipids content determination in the manufacturing process. The FTIR method, which we developed and validated, meets all criteria for the in-process control method, allowing, if necessary, making adjustments in the production process.

TUMOR CELL GLUTAMINOLYSIS MAY EXPLAIN ENHANCED TUMOR DOXORUBICIN RELEASE FROM DOXIL®

Lisa Silverman, Yechezkel Barenholz
Hadassah Medical School, Hebrew University, Israel

Purpose: To determine the mechanism by which drug-loaded pegylated nano-liposomes such as Doxil®, which accumulate at the tumor site due to the EPR effect, release drug at a level sufficient for therapeutic efficacy, and to investigate how the tumor microenvironment is involved in this release process.

Materials and Methods: We studied the physicochemical properties of the pegylated liposomal doxorubicin (PLD) Doxil and its generic version, DOX-NP, in contrast to two other types of liposomes, one of which is also a doxorubicin-loaded nano-liposome but with a different loading method, and the other, a much larger multi-lamellar vesicular vesicle (LMVV). We extensively studied their release kinetics under varying conditions in vitro that imitate the tumor microenvironent.

Results: Our results show that low-molecular-weight species in the tumor microenvironment play a major role in doxorubicin release. Substantial release rates occur for both nano-liposomes used in this study, irrespective of the type of transmembrane ion gradient driving the drug loading. However, LMVV loaded with doxorubicin exhibit an almost 3-fold slower release rate, pointing to the contribution of the nano-volume of the nano-liposomes to the rate of drug release in the tumor.

Conclusions

- Tumor microenvironment plays a major role in the release of amphipathic weak base drugs from liposomes in which these drugs are remote loaded by transmembrane gradient of either ammonium or hydrogen ions.
- Liposomal trapped volume is an important factor in drug release rate. Liposomes with a larger trapped volume have a much lower drug release rate than nano-liposomes.

PROTEIN PRODUCING PARTICLES FOR ONSITE DRUG DELIVERY

Janna Shainsky, Dikla Vardi, Avi Schroeder
Chemical Engineering, Technion, Israel

Proteins have great therapeutic importance. Large scale industrial plants and small scale laboratory experiments depend on the efficiency of protein production systems.

Previously, we developed nanoparticles that act as semi-synthetic cells – capable of producing proteins autonomously in response to a physical stimulus [1]. These nanoparticles can be injected into the body and then triggered to produce a protein of interest onsite. Herein, we sought to improve our understanding of these synthetic particulate systems. Specifically, we studied how different fabrication conditions affect the activity of the molecular machines (such as, ribosomes and RNA polymerase) that were loaded into the particles. Furthermore, we sought to improve the protein production efficiency of these systems per unit volume by reformulating the internal 'mix' with a new and highly potent composition of amino acids, energy regenerating compounds, ribosomes etc [2]. Finally, the system was designed to respond to relevant endogenous biomarkers by introducing a repressor key in the encoding gene. Specifically, we incorporated LacI, TetR and riboswitch encoding genes on the plasmid of interest. That enables controlled production of protein of interest in defined environment.

Responsive synthetic systems with biological functions provide better understanding of biological phenomena and may help advance medicine.

REFERENCES

1. Schroeder, A. *et al.* Remotely Activated Protein-Producing Nanoparticles. *Nano Letters* **12**, 2685-2689, (2012).

2. Shainsky-Roitman, J., Schroeder, A. & Shoham, Y. METHODS AND KITS FOR CELL-FREE PROTEIN SYNTHESIS. (2014).

RELEASE PROFILING AS A SCREENING TOOL IN DEVELOPMENT OF NOVEL LIPOSOMAL BUPIVACAINE FORMULATIONS

Katia Ioffe[1], Genia Levinton Shamuilov[1], Nofar Keren[1], Doron Friedman[1,2], Chezy Barenholz[2], Dima Shamrakov[1]

[1]*LipoCure Ltd, Jerusalem Biotechnology Park, Hadassah, Israel*

[2]*Laboratory of Liposome and Membrane Research, IMRIC, Hebrew University-Hadassah Medical School, Israel*

In-vitro dissolution testing is one of the most important tools used in pharmaceutical formulation development. It serves the following main purposes: screening and selection of candidate formulations, prediction of *in-vivo* bioavailability, and batch-to- batch reproducibility and the quality control.

The long acting anesthetic injectable formulation based on large (~10µm) multi-vesicular liposomes loaded with bupivacaine in different hydrogel matrixes aims for slow controlled drug release.

Standard USP dissolution Apparatus 2, equipped with mini-paddles/mini-vessels assemblies, was used for *in-vitro* release testing. An in-house-designed enhancer cell with 0.2 µm membrane allowed close simulation of subcutaneous depot injection.

Release rate is a function of lipids composition and concentration, as well as the drug loading conditions. Embedding the liposomes in hydrogels was shown to have essentially no effect on the drug release. The hydrogel's role is mainly confinement of liposomes in the injection site. Changing the formulation variables enabled us to achieve different release rates, starting from fast release resembling the free drug performance, to the slow drug release similar to that of a commercially-available brand product.

THYROID HORMONES-αvβ3 INTEGRIN-MEDIATED PATHWAY IN OVARIAN CANCER: A VALID TARGET FOR INHIBITION

Elena Shinderman[1,2], Keren Cohen[1,2], Martin Ellis[1,3], Aleck Hercbergs[4], Paul J Davis[5], Osnat Ashur-Fabian[1,2]

[1]*Translational Hemato-Oncology laboratory, Meir Medical center, Israel*
[2]*Sackler Faculty of Medicine, Department of Human Molecular Genetics and Biochemistry, Tel-Aviv University, Israel*
[3]*The Hematology Institute and Blood Bank, Meir Medical center, Israel*
[4]*Radiation Oncology, The Cleveland Clinic Foundation, USA*
[5]*Department of Medicine, Albany Medical College, USA*

Background and aims: Ovarian carcinoma, the fifth most common cancer in women, remains highly resistant and novel treatments are urgently needed. αvβ3 integrin membrane receptor is over-expressed on many cancer cells, including ovarian cancer, recognizing RGD motifs on extra cellular proteins. Recently, a novel non-RGD receptor site has been described on this integrin, binding mainly thyroid hormones (T3 and T4) and activating the MAPK pathway. Tetrac, a non-agonistic T4 derivate, blocks this activation. We aimed to study the effects of activating/blocking this novel thyroid-αvβ3 axis, for the first time in ovarian cancer models.
Methods: Ovarian cancer cell lines (OVCAR-3, SKOV-3 and A2780) were grown with/without T3/T4 (0.1nM-1μM) in the presence/absence of tetrac/RGD (10nM-100μM) or αvβ3 blocking antibody (0.001-1μg/ml). Experiments were analyzed by several methods: cell count (FACS, CyQuant), viability (WST-1), proliferation (BrdU), cell cycle (FACS, PI), cell death (Annexin-PI, FACS), αvβ3 expression (FACS, IF) and MAPK signaling (Westerns and IF).
Results: We show that supra-physiological T3 (1nM) and physiological T4 (100nM) concentrations increase cell viability and proliferation in ovarian cancer cells. This increase was accompanied by a reduction in cell death. Tetrac efficiently reversed the proliferative effects of thyroid hormones and induced cell death, in a dose dependent manner. Following T3/T4 treatments, a quick and long lasting MAPK pathway activation was observed. This action was blocked by tetrac as well as by RGD and αvβ3 blocking antibody, indicating involvement of this integrin.
Conclusions: Thyroid hormones exhibit growth-factor qualities in ovarian cancer cells which appear to be αvβ3-mediated. Therefore, we propose that blocking this axis is a novel treatment paradigm which we intend to further explore in this malignancy.

POSTER #77

SIZE AND SHAPE OF NANO-DRUGS AND THEIR EFFECT ON CANCER TREATMENT

Moria Barlev-Gross, Erez Koren
Hadassa medical school, Hebrew University, Israel

The design of nano-drugs of precise size, shape and morphology distributions is a crucial parameter for nano-drug performance, as it may influences their physical properties, active pharmaceutical ingredient *in vitro* release, *in vivo* pharmacokinetics, biodistribution, toxicity, therapeutic efficacy and consequently its therapeutic index.

In this work, we have prepared liposome-based nano-drugs in controlled size and morphology distributions, all having identical lipid composition. By remote-loading with increasing drug levels, liposome shape changed from spherical to prolate ellipsoid with increasing axial ratio. The higher the drug-to-lipid ratio, the larger is the axial ratio. These nano-liposomes were characterized using a variety of techniques, including dynamic light scattering (DLS), quantitative cryo-transmission electron microscopy (cryo-TEM) and solution X-ray scattering analysis. Our cryo-TEM analysis determined shape parameters including axial ratio distributions, liposomal volume distributions, as well as the physical shapes of the drug in the intra-liposomal aqueous phase. A drug-release study was performed in order to measure the effect of encapsulated crystalized doxorubicin on drug-release profile. Using conditions that imitate tumor microenvironment (See Silverman *et al.* poster), when tested in 50mM ammonium sulfate (at 37^{o}C), there were non-significant differences in drug-release between the various doxorubicin-loaded liposomal formulations. In human plasma and in sodium sulfate, saline and 10mM histidine solutions, where drug release is minimal, it had no effect at all. An *in vivo* study using an ovarian cancer mouse model found that all drug-loaded nano-liposomes (with spherical to prolate ellipsoid morphology) were equivalent with respect to efficacy, tumor size and animal weight parameters.

Our data may enable us to define the range in which therapeutic efficacy of 80-150 nm length liposomal particles having different morphology properties act similarly. Understanding how size and morphology can affect the biodistribution of IV-injected particles is of major importance, both for rational design of drug delivery systems and for regulatory standardization.

NON-RGD-BASED STRATEGIES TO TARGET THE THYROID HORMONE-αvβ3 INTEGRIN AXIS: LESSONS FROM MYELOMA CELLS

Keren Cohen[1,2], Martin Ellis[1,3], Paul J Davis[4], Aleck Hercbergs[5], Osnat Ashur-Fabian[1,2]
[1]*Translational Hemato-Oncology Laboratory, Meir Medical Center, Israel*
[2]*Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Israel*
[3]*Hematology Institute and Blood Bank, Meir Medical Center, Israel*
[4]*Department of Medicine, Albany Medical College, USA*
[5]*Radiation Oncology, Cleveland Clinic, USA*

Introduction: Integrin αvβ3 is a plasma membrane protein expressed/activated generously in tumor cells and dividing blood vessel cells. The extracellular domain of αvβ3 has an Arg-Gly-Asp (RGD) recognition site that enables the integrin to bind specific extracellular matrix (ECM) proteins. Recently, a receptor for thyroid hormones (T4 and T3) has been described on αvβ3 that is distinct from the RGD recognition site and has been shown to mediate proliferative effects on solid tumor cells via the MAPK pathway. We have recently shown that similar action occurs in multiple myeloma (MM). Tetraiodothyroacetic acid (tetrac),a naturally-occurring deaminated T4 analog can block these actions. In this report, we define mechanisms of tetrac action in MM cells.
Methods: MM cell lines (CAG/RPMI/8226/ARK/ARP-1/U266) and primary cultures of marrow cells from MM patients were grown with T3/T4 and with/without tetrac (100nM-50μM). Harvested cells were subjected to the following analyses: cell counts (FACS, CyQuant), viability (WST-1), cell cycle (FACS, PI), cell death (Annexin-PI ± pan-caspase inhibitor, ZVAD, FACS; cleaved caspase 3, cleaved PARP, western blots), DNA damage response (pATM and PARP, western blots) and transcription of apoptosis-relevant genes (RT-PCR).
Results: Early (4-8 hours) and late (24 hours) effects of tetrac were observed. In the early phase, tetrac reduced cell proliferation (p0.05) and induced DNA damage response (ATM and PARP) and apoptosis (cleaved caspase 3 and cleaved PARP). After 24 hours, tetrac reduced significantly (p0.05) cell number and cell viability and induced apoptotic cell death with a parallel increase in pro-apoptotic gene expression.
Conclusions: Multiple myeloma has a poor prognosis despite a number of therapeutic approaches. The thyroid hormone non-RGD binding site on αvβ3 in myeloma cells appears to be a new target for chemotherapy via tetrac.

RE-SENSITIZATION OF CHEMO-RESISTANT CANCER CELLS USING SIRNA-DELIVERING NANOMEDICINE

Tal Zur, Ronit Satchi-Fainaro
Physiology and Pharmacology, Tel Aviv University, Israel

Resistance to chemotherapy is one of the main challenges in the fight against cancer. Chemotherapeutic efficacy is restricted by acquired resistance to treatment and by toxic side effects which severely limit the administered maximal tolerated dose (MTD). Gene-silencing of resistance-implicated genes through delivery of siRNA to tumor cells, may enable to partially re-sensitize resistant cancers to chemotherapy. We are currently exploiting two polymeric nano-carriers for siRNA delivery, to be administered prior to chemotherapy. Each of these positively-charged nano-carriers will be electrostatically complexed with siRNA targeting a resistance-related gene, which silencing may re-sensitize chemo-resistant cells. We have previously developed in our laboratory several types of chemo-resistant cancer models. Our models consist of four pairs of cell lines from different types of cancers - each pair comprised of a parental cell line and a derivative which is resistant to a specific chemotherapeutic agent. In each model, we have chosen a number of gene-targets that have been previously implicated in chemo-resistance. Among those, we have selected the most appropriate one in each model by confirming and quantifying their overexpression in the resistant cell lines using RT-PCR mRNA quantification, and Western Blot semi-quantification of protein levels. We are using siRNAs designed to silence these overexpressed targets. In this work, we are evaluating *in vitro* the ability of our nano-carriers complexed to the appropriate siRNA to re-sensitize cancer cells to the chemotherapeutic agent to which they have become resistant to. We are evaluating the ability of the nanocarriers to silence the relevant gene and re-sensitize the cells to chemotherapy in all four models in order to determine the universality of our approach.

X-ray Scattering: A Powerful Tool to Aid the Design of Drug-Loaded Liposomes

Yechezkel Barenholz[2], **Yaelle Schilt**[1], Xiaohui Wei[2], Tal Berman[2], Erez Koren[2], Uri Raviv[1]

[1]*Institute of Chemistry, The Hebrew University of Jerusalem, Israel*

[2]*Laboratory of Liposome and Membrane Research, IMRIC, Hebrew University-Hadassah Medical School, Israel*

During the last 40 years, research on liposomes as drug carriers has been getting increasing attention and has matured into more than 15 FDA-approved liposomal drugs. The FDA approval is related to the significant improvement in therapeutic index, due to large changes in drug pharmacokinetics and biodistribution. In the case of tumors, this is due considerably to the passive targeting to the tumor tissue. The liposomal anti-cancer 80-90nm nano-drug Doxil/Caelyx, approved by the FDA in 1995 and in clinical use since then, is a good example of such passive targeting, demonstrating the importance of the physicochemical properties of a nanoparticle acting as drug-delivery system to the nano-drug's performances (Barenholz, J. Contr. Release (2012). That explains why, in order to approve a liposomal drug, the FDA requires in-depth characterization of the physicochemical features of the liposomes [see Draft Guidance on Doxorubicin-Hydrochloride: www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/UCM199635.pdf].

For example, long circulation of the drug-loaded liposomes in the blood stream, a crucial point for their efficiency is achieved by the incorporation of polyethylene glycol covalently attached to phosphoethanolamine (DSPE-PEG) into the liposomal constituents, giving rise to sterically stabilized liposomes. The conformation of the PEG-layer is a key parameter of the nano-drug's performance; however this information is not easily obtained as most methods are not sensitive for measuring the PEG layer. The physical state of the drug inside the liposome, which is critical for high therapeutic efficacy and low toxicity, is another example.

By combining high-resolution solution X-ray scattering experiments and advanced analysis tools developed and validated in our lab, we studied various features of the pegylated liposomal doxorubicin nano-drug, including the effect of DSPE-PEG mole ratio. The scattering curves of the liposomal doxorubicin revealed the detailed structure and the aforementioned crucial parameters. These data beautifully complement the information obtained from cryo-TEM images of the same structures.

ULALA (ULTRA-LONG-LOCAL-ANESTHETICS) BASED ON BUPIVACAINE REMOTE-LOADED INTO LARGE-MULTI-VESICULAR-VESICLES (BUPISOME) EMBEDDED IN HYDROGEL TO FORM BUPIGEL

Rivka Cohen, Hiba Kanaan, Yechezkel Barenholz
Laboratory of Liposome and Membrane Research, IMRIC, Hebrew University-Hadassah Medical School, Israel

Bupisome and Bupigel[1] ULALA formulations demonstrate slow and controlled drug release leading to low toxicity and prolonged analgesia. Therefore they hold great promise for management of acute pain after surgery or trauma. Clinical trials with Bupisome reveal a 15 fold prolonged analgesia in humans compared to free bupivacaine[2]. However the Bupisome did not have the required long term stability upon storage. To overcome this major deficiency we embedded an improved Bupisome in alginate cross linked hydrogel to fabricate the Bupigel.

Bupisomes and Bupigel were screened in in-vitro drug release assay at 4^0C to determine stability upon storage and at 37^0C to predict analgesia efficacy. Only Bupisome which have a drug to phospholipid mole ratio 1.0 were selected. Fabrication of suitable large multi vesicular vesicles (LMVV), based on phospholipid having their Tm 37^0C, together with 40mole% cholesterol is a prerequisite[1], resulting in "liquid ordered" lipid bilayer. In addition, Bupigel was stored in 0.5% - 2%- aqueous "free" bupivacaine to prevent loss of drug from the liposomes and increasing storage stability at 4C° concomitantly with preventing a lag period in the analgesia upon local administration. Analgesia duration was assessed using the pain model of Swiss Webster male mice[1,2].

All BupiSome and Bupigel formulations tested showed much superior and prolonged analgesia than free bupivacaine, with Bupigel being superior to Bupisome. An additional advantage of Bupigel is that before injection the excess free bupivacaine can be easily removed. Further experiments are underway to determine the toxological outcomes of these formulations in order to enable clinical trials with Bupisome.

REFERENCES

[1]Cohen et al. 2012, JCR 160, 346-352

[2]Grant et al.2004, Anesthesiology 101, 133-137

REGULATION OF HEPATIC ACUTE PHASE RESPONSE BY GENE SILENCING USING ANIONIC POLYPLEXES OF STAT3 siRNA

Edan Elovic
Biotechnology Engineering, Ben Gurion University, Israel

Acute phase response is an orchestrated response to injury, infection or inflammation and takes part in the restoration of homoeostasis. Cytokines, such as the IL-6, are important mediators of the acute phase response and when their action is uncontrolled and prolonged, it could be potentially harmful, often leading to a chronic phase reaction. Pathogenesis of the acute phase response is linked to constitutive activation of Signal transducer and activator of transcription 3 (STAT3), making it a novel therapeutic target for gene silencing using small interfering RNA (siRNA). Efficient systemic delivery necessitates the use of carrier to protect siRNA molecules until reaching their site of action and to facilitate cell penetration**.** In my research, I investigated whether the acute phase response can be controlled by gene silencing using anionic polyplexes containing siRNA for STAT3. The nano-sized anionic polyplexes, developed in our lab, consist of the bio-inspired hyaluronan sulfate polymer and siRNA mediated by calcium ions. Using confocal microscopy, I showed efficient cellular uptake of the anionic polyplexes into HepG2 cells and accumulation in the cytoplasm. Moreover, I obtained up to 90% silencing of *stat3* gene by quantitative Real-Time PCR (qPCR). Finally, I showed that effective silencing of *stat3* prevented IL-6-induced expression of acute phase genes. In conclusion, siRNA delivery platform based on hyaluronan-sulfate polyplexes was found effective in acute phase cellular model, suggesting its potential as a novel treatment for pathological inflammatory conditions.

THE INNER DOCTOR - TREATING THE METABOLIC SYNDROME FROM WITHIN

Roni Itzhaki
Biotechnology Engineering, Ben Gurion University, Israel

Western modern lifestyle brought us a lot of wonderful innovations, but also many problems. Today, about 25% of the adult world population suffers from the Metabolic Syndrome. This syndrome is a cluster of dangerous medical conditions, including: obesity, insulin resistance, high blood triglycerides and more. These medical conditions are interconnected and are a result of complex metabolic pathways, meaning most traditional drugs used to treat them have undesired side effects. I intend to utilize synthetic biology in order to develop novel strategies for the treatment of the Metabolic Syndrome. By addressing the causes of the syndrome rather than its symptoms, and by doing so in several different fronts, I hope to present a safe and better way to improve global health.

siRNA NANOPARTICLES TARGETED TO THE EXTRA CELLULAR MATRIX FOR CANCER

THERAPY

Meital Naim[1], Mirjam M. Nordling-David[1], Arie Dagan[1], Zvika Granot[2], Gershon Golomb[1]

[1]*Institute for Drug Research, Faculty of Medicine, Israel*

[2]*Institute for Medical Research, Faculty of Medicine, Israel*

Osteolytic metastasis is a leading event in the course of breast cancer progression. At this stage of the disease, there is no curative treatment available yet. Recently, a set of genes has been identified that is associated with the generation of skeletal metastatic lesions from primary breast cancers. It includes the phosphoglycoprotein osteopontin (OPN), which is a constituent of the skeletal extracellular matrix (ECM). We hypothesized that siRNA treatment against OPN could reduce lytic skeletal metastases.

Since the therapeutic application of siRNA is limited due to short half-life, large molecular size and high negative charge density, successful siRNA delivery relies on a suitable delivery system, which would protect the molecules from degradation and enable their internalization into the target cells. Non-viral gene carriers, polymeric and lipoidic-based nanoparticles (NP), have been shown as a promising gene delivery method for the treatment of a variety of inherited and acquired diseases. By targeting the NP it is possible to specifically localize and target the drug cargo, and at the same time providing a protected and prolonged drug release, thus minimizing side effects.

Our targeting moiety is a 25AA peptide sharing the same binding moiety as Apo-B100 possessing high affinity to proteoglycans in the extracellular matrix. *In-vitro* binding studies show enhanced binding of both polymeric-and lipoidic-based targeted NP to proteoglycans in ECM compared to non-targeted NP. Preliminary *in-vivo* results in a 4T1 mammary carcinoma mice model show a significantly enhanced uptake of targeted NP to the cancerous lung.