THE 8TH ANNUAL MEETING OF THE ISRAELI CHAPTER OF THE CONTROLLED RELEASE SOCIETY (ICRS)

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

September 5-7, 2012

Hacienda Forestview Hotel, Maalot, Israel

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PROGRAM

Wednesday – September 5, 2012

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

Rooms check-in at 15:00 pm

13:00 **LUNCH**

14:00 - 14:15 **Welcome and introductory remarks**
Ronit Satchi-Fainaro, ICRS President
Ehud Gazit, The Chief Scientist, The Ministry of Science and Technology, Israel

14:15 - 16:20 **SESSION 1**
NANOMEDICINES FOR CANCER THERAPY
Chairpersons**:** ***Alberto Gabizon and Yechezkel Barenholz***

14:15- 15:00 **KEYNOTE PRESENTATION**
"Nanomedicines: Polymer Therapeutics- the end of the beginning"
Ruth Duncan
Cardiff, UK and CIPF, Valencia, Spain

15:00 – 15:20 Avi Schroeder (MIT, Technion)- **"Focused cancer treatment: From drug delivery systems to programmed nano robots**"

15:20- 15:40 Ayelet David (BGU)- "**Stimulus-sensitive copolymer-conjugates for triggering intracellular drug penetration"**

15:40- 16:00 Simon Benita (HUJI)**- "Targeted drug loaded nanoparticles for improved cancer treatment"**

16:00- 16:20 Itai Benhar (TAU)- **"Studying design principles for bi-specific antibodies"**

16:20- 16:50 **COFFEE BREAK**

16:50- 19:00 **SESSION 2**
TARGETING STEM CELLS AND REGENERATIVE MEDICINE
Chairpersons**:** ***Smadar Cohen and Rosa Azhari***

16:50- 17:10 Marcelle Machluf (Technion)- **"Cell derived vesicles: turning natural targeting to smart drug delivery systems"**

17:10- 17:30 Tal Dvir (TAU)- "**Delivering instructive cues and imaging biomolecules to 3D engineered tissues"**

17:30- 17:50 Boaz Tirosh (HUJI)- "**The unfolded protein response as a regulator of drug induced liver injury"**

Wednesday – September 5, 2012 *(Continued)*

18:00- 19:15 ***Short presentations (15 x 5 min) of selected posters from submitted abstracts:***

1. Gili Bisker (Technion)- Controlled release of Rituximab from gold nanoparticles for phototherapy of malignant cells

2. Eliahu Heldman (BGU)- Brain delivery of proteins using bolaamphiphilic nano-sized vesicles: mechanisms and efficiency

3. Ravit Edelman (Technion)- Hyaluronic acid based quadrugnostic nanoparticle for cancer therapy

4. Anat Eldar-Boock (TAU)- Overcoming paclitaxel resistance with anti-angiogenic and anticancer RGD-bearing nanomedicine

5. Adi Golani (BGU)- Polyion complexes for gene delivery to dendritic cells

6. Neta Zilony (BIU)- A novel biolostic technology for controlled delivery of nano-structured porous Si drug carriers

7. Talia Shekhter (TAU)- Novel somatostatin analog conjugates for pancreatic cancer detection

8. Mirjam M. Nordling-David (HUJI)- Targeted siRNA liposomes and nanoparticles

9. Anna Scomparin (TAU)- Folated drug deliverysystems of doxorubicin: a comparative study

10. Wahid Khan (HUJI)- Pharmacokinetic and efficacy study of cisplatin formulated in a new injectable polymer

11. Nadav Ben-Dov (TAU)- Crossing the barrier: New pathway for delivering molecules across the cell's plasma membrane

12. Nour Karra (HUJI)- Design and evaluation of drug loaded cetuximab immunonanoparticles prepared using a novel linker molecule

13. Brenda Laster (BGU)- Simultaneous chemotherapy and radiotherapy of tumors

14. Alexander Adriyanov (HUJI)- Anticancer therapy based on nanodrug chemotherapy combined with thermal ablation

15. Yaelle Felsen (HUJI)- An Improved Pegylated Liposomal Doxorubicin with Significantly Lower PPE than Doxil®

20:30 **COCKTAIL RECEPTION AND POSTER SESSION**

Thursday – September 6, 2012

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

8:30-10:15 **SESSION 3**
NANO-PARTICULATED DRUG DELIVERY SYSTEMS
Chairpersons**:** ***Rimona Margalit and Shimon Amselem***

8:30 - 9:15 **KEYNOTE PRESENTATION**
The next step in drug targeting: Inside cells and to individual organelles
Vladimir Torchilin
Northeastern University, MA, USA

9:15-9:35 Chezy Barenholz (HUJI)**- "PEGylated Nano-liposomes loaded with amphipathic weak acid glucocorticosteroids for successful systemic treatment of animal models for Rheumatoid arthritis (RA), multiple sclerosis (MS), cerebral malaria (CM) and cancer"**

9:35-9:55 Alberto Gabizon (Shaarei Zedek)- "**A novel formulation of liposome co-encapsulated alendronate and doxorubicin for cancer therapy**"

9:55-10:15 Dan Peer (TAU)- **"Gagomers: Novel Safe Particle Clusters for Systemic Delivery of Therapeutic Payloads"**

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

10:45-11:05 Mimi Kaplan (Ministry of Health)- **"Implementation of Quality by Design – a new approach to drug development"**

11:05-11:25 Rimona Margalit (TAU)- **"Acute and chronic inflammations treated by steroid, NSAID and antioxidant drugs encapsulated alone or in combination in biomaterial-based bioadhesive carriers"**

11:30-13:30 **SESSION 4**
INDUSTRIAL VIEW ON CONTROLLED RELEASE OF BIOACTIVE MATERIALS
Chairpersons**:** ***Ittai Harel and Amira Zeevi***

11:30-12:00 Aharon Schwartz- **"Targeted therapy = Personalized Therapy?"**

12:00-12:15 Gal Ehrlich (Ehrlich & Fenster, Patent Attorneys)**- "IP Strategy for Drug Development"**

12:15-12:30 Noam Emanuel (PolyPid Ltd.)- **"BonyPid^{TM}: A lipid- and polymer-based novel drug delivery sytem: physicochemical aspects and therapy"**

Thursday – September 6, 2012 *(Continued)*

12:30-13:30 **INDUSTRIAL DISCUSSION PANEL:**
Moderator: Simone Botti (Merck Serono)
Panel members: Aharon Schwartz, Amira Zeevi (Perrigo), Sigal Blau (Teva Pharmaceuticals**),** Adrian Gilbert (Teva Pharmaceuticals), Ittai Harel (Pitango), Gal Ehrlich (Ehrlich & Fenster), Noam Emanuel (PolyPid), Adel Penhasi (DeGama & SPAI Group), Shimon Amselem (Nextar ChemPharma Solutions Ltd.), Yaniv Dolev (Moebius Medical Ltd.).

13:30-15:00 **LUNCH FOR ALL PARTICIPANTS AND POSTER VIEWING**

15:00-17:00 **SESSION 5**
DRUG DELIVERY ISSUES IN INFECTIOUS AND RARE DISEASES
Chairpersons**:** ***Jean-Paul Lellouche and Yechiel Shai***

15:00- 15:20 Shula Michaeli (BIU)- **"Nano-Silencing from Trypanosomes to Man"**

15:20- 15:40 Yechiel Shai (WIS)- **"Targeting and lysis of bacteria, fungi and cancer by host-defense-like membrane active peptides and nano-particle lipopeptides "**

15:40- 16:00 Avi Domb (HUJI)- **"Crystallization and release of rapamycin from metallic stents"**

16:00-16:20 Meital Reches (HUJI)- **"Self-assembly of biomolecular necklaces"**

16:20-17:05 ***Short presentations (9 x 5 min) of selected posters:***

1. Yael Lupu (Technion)- Elucidating the mechanism of interactions between mesenchymal stem cell derived nano-vesicles and cancer cells

2. Yosi Shamay (BGU)- Controlled penetration of HPMA-R8 copolymers into tumors using biodegradable polyanions

3. Aharaon Azaguri (BGU)- The mechanism of ultrasound effect on chorioamnion membrane mass transport

4. Naama Toledano Furman (Technion)- Stem cell derived nano-vesicles, a new delivery platform for cancer targeted therapy

5. Dina Polyak (TAU)- Development of nano-scaled polymeric carriers for integrin-assisted doxorubicin delivery

6. Oren Giladi (HUJI)- Targeted radiolabeled immune-nanoparticles for treatment of HER-2/neu positive cancers

Thursday – September 6, 2012 *(Continued)*

7. Yossi Kam (HUJI)- Detection of endogenous K-Ras mRNA in living cells by molecular beacons

8. Shoshy Mizrahy (TAU)- Hyaluronan coated nanoparticles: the influence of them Mw on CD44-Hyaluronan interaction and immune response

9. Hadas Skaat (BIU)- Magnetic scaffolds enriched with bioactive nanoparticles for tissue engineering. **Winner of the Barenholz Prize.**

17: 05-17:30 **COFFEE BREAK**

17:30-19:00 **SESSION 6**
SELECTED ROUTES OF ADMINISTRATION
Chairpersons: ***Amnon Hoffman and Gershon Golomb***

17:30 -17:50 Avri Rubinstein (HUJI)**- "Mucosal targeting of biomarkers for real-time diagnostics of GI malignancy by drug delivery tools"**

17:50-18:05 Yoav Livney (Technion)- **"Quadrugnostic nanoparticles for cancer therapy"**

18:05-18:20 Gershon Golomb (HUJI)**- "Liposomal bisphosphonate mechanism of action in inhibiting restenosis: Effect on monocytes subpopulations"**

18:20-18:35 Dvir Yelin (Technion)- **"Release of antibodies from gold nanospheres during short-pulse laser phototherapy"**

18:35-18:50 Sigal Blau (Teva Pharmaceuticals)**- "Gastric retentive oral dosage forms –when reality met theory"**

18:50-19:20 ***Short presentations (6 x 5 min) of selected posters from industry abstracts:***

1. Hilla Epstein-Barash (Entrega Inc.)- "Orally bioactive mucoadhesive intestinal polypeptide delivery systems"

2. Shimon Amselem (Nextar ChemPharma Solutions Ltd.)- "Effective sublingual delivery of flumazenil formulation for reversing the residual sleepiness effect of hypnotic drugs"

3. Salit Tzaban (Protalix Biotherapeutics)- "Plant cells as oral delivery vehicle of human recombinant glucocerebrosidase for the treatment of Gaucher disease"

4. Yaniv Dolev (Moebius Medical Ltd.)- "Liposomes as lubricants, a new approach for osteoarthritis – Ongoing trials"

Thursday – September 6, 2012 *(Continued)*

5. Joseph Wyse (Patents, Dr. Eyal Bressler Ltd.,)- "Patent protection for innovatins in anti-angiogenic therapy"

6. Tal Sarid (Dr Golik)- Break-through in the processing and controlling of biodegradable micro-capsules as drug carriers

20:30 **GALA DINNER**

- **Presentation of the ICRS Prize for Outstanding Achievements in Controlled Release to Prof. Simon Benita**
- **Presentation of the 2012 Barenholz Award to Hadas Skaat sponsored by Professor Chezy Barenholz**
- **Presentation of the 2012 Best Student Poster Awards:**
  First prize: Award sponsored by Teva Pharmaceuticals Ltd.
  Second prize: Award sponsored by Dexcel Pharma Ltd.
  Third prizes: Awards sponsored by Unipharm Ltd. and Silicol Scientific Equipment Ltd.

Friday – September 7, 2012

Check-out at 11:00 am

8:30-10:35 **SESSION 7**
THERANOSTIC NANOTECHNOLOGIES FOR PERSONALIZED MEDICINE
Chairpersons: ***Ehud Gazit and Roey Amir***

8:30 - 9:15 **KEYNOTE PRESENTATION**
Molecular trees: From the test tube towards biomedical applications
Rainer Haag
Frei University Berlin, Germany

9:15-9:35 Rachela Popovtzer (BIU)- **"Gold nanoparticles as computed tomography imaging contrast agents"**

9:35-9:55 Thomas L. Andresen (Technical University of Denmark)- **"Liposome imaging agents for personalizing treatment with nanocarriers"**

9:55-10:15 Eylon Yavin (HUJI)**- "Peptide nucleic acids (PNAs) in cancer diagnosis"**

10:15- 10:35 Galia Blum (HUJI)- **"Quenched activity based probes for simultaneous detection and therapy of cancer"**

10:35-11:00 **COFFEE BREAK**

11:00-12:30 **SESSION 8**
NOVEL BIOMOLECULAR SELF-ASSEMBLED SYSTEMS FOR DRUG DELIVERY
Chairpersons: ***Yaron Dekel and David Stepensky***

11:00- 11:20 Niv Papo (BGU)- "**Directed evolution of affinity scaffolds for drug delivery and biomedical diagnostics"**

11:20-11:40 Yael Shaked (Dr Golik)**- "Benefits of different characterization techniques for drug delivery application"**

11:40- 12:00 Roey Amir (TAU)**- "Hybrid PEG-dendritic scaffolds as nano-carriers for drug and gene delivery"**

12:00-12:20 Ehud Gazit (TAU)- **"The mechanism of association and technological applications of self-assembled peptide nanostructures"**

12:20-12:30 **Concluding remarks**
Ronit Satchi-Fainaro

12:30- 13:30 **FAREWELL LIGHT LUNCH FOR ALL PARTICIPANTS**

ABSTRACTS

NANOMEDICINES: POLYMER THERAPEUTICS- THE END OF THE BEGINNING

Ruth Duncan
Polymer Therapeutics Lab, c/o CIPF, Spain

A growing number of polymer therapeutics have gained market authorisation for routine clinical use and/or are in clinical development (Duncan R., Curr. Opin. Biotechnol. 22: 1-10, 2011).
The fact that the Regulatory challenges of the emerging 'follow-on/generic' products are under discussion is testament to the maturity of the field. It is clear that lessons learnt from >3 decades of clinical studies (Duncan R., Adv. Drug Del. Rev., 61: 1131-1148, 2009; Duncan R. & Vicent M.J., Adv. Drug Del. Rev., 62: 262-272, 2011), recent advances in cell biology/the understanding of endocytosis in health and disease (Duncan R. & Richardson S.W.C., Mol. Pharmaceutics, in press, 2012), and emergence of new technologies across the nanosciences are leading to a paradigm shift in design and development of polymer therapeutics/nanomedicines (Duncan R. & Gaspar R., Mol. Pharmaceutics, 8: 2101-2141, 2011). Embracing these opportunities will bring improved conjugate design and selection of the most appropriate patients for therapy. Our recent studies have focused on development of techniques for validation of preclinical models, design of bioresponsive conjugates to promote tissue repair and as anticancer agents, and the design of polymer-coiled-coil conjugates as therapeutic agents.

Keywords: Nanomedicine, Polymer Therapeutics, Endocytosis

FOCUSED CANCER TREATMENT: FROM DRUG DELIVERY SYSTEMS TO PROGRAMMED NANO ROBOTS

Avi Schroeder
Chemical Engineering, Technion, Israel

Metastasis is the cause of 90% of cancer deaths. In many cases, by the time a primary tumor is detected, subsets of malignant cells have already disseminated to other locations in the body seeding the spread of the disease. Nanoparticles have many potential benefits for treating metastatic cancer, including the ability to transport complex molecular cargoes, as well as targeting specific cell population. The lecture will describe the development of nanoscale lipid vesicles, loaded with drugs and small interfering RNA (siRNA), that can be remotely triggered to release their payload at a focused tumor site. The evolution of these vesicles into *programmed nano robots*, unique particles that have an internal capability of synthesizing proteins, and their promise for treating metastasis, will be addressed. Furthermore, the construction of a miniature electronic device developed for enhancing the delivery of drugs to specific tissues will be described.

Keywords: protein, metastasis, liposome

STIMULUS-SENSITIVE COPOLYMER-CONJUGATES FOR TRIGGERING INTRACELLULAR DRUG PENETRATION

Ayelet David

Pharmacology, Ben-Gurion University of the Negev, Israel

Cell-penetrating peptides (CPPs) have been shown to promote intracellular delivery of a broad variety of cargoes, including various nanoparticulate pharmaceutical carriers, both *in vitro* and *in vivo*. However, the lack of cell-specificity limits their *in vivo* use for drug delivery applications. We exploited two novel strategies to overcome the lack of cell specificity of CPPs to create drug delivery systems with improved tumor-targeting ability. The cationic residues of the CPP sequence were caged or neutralized with a photo-cleavable molecule or polyanionic sequences, respectively. An external or environmental stimuli (e.g. UV-light illumination or augmentation of counter-polycations, respectively) has been applied to enhance intracellular penetration of the CPP-containing polymer-drug conjugates. The light activated penetratin sequence promoted an effective intracellular delivery and antitumor activity of the pro-apoptotic peptide $_{D}(KLAKLAK)_2$ into 80% of the target cells following 10-minutes of UV-light illumination. The conditional release of the octa-arginine (R_8) that was effectively masked with counter polyanions, following the addition of an arginine-rich polycationic counterpart, improved the cytotoxicity of the polymer-conjugate bearing doxorubicin (P-R_8-DOX) towards endothelial and cancer cells, both *in vitro* and *in vivo.* The survival of mice with established lung metastases was remarkably prolonged after a single administration of P-R_8-DOX in complexation with polyanionic sequences.

Keywords: polymer-drug conjugates, CPP, HPMA copolymer, light illumination, polyion complexes

TARGETED DRUG-LOADED NANOPARTICLES FOR IMPROVED CANCER TREATMENT

Simon Benita, Taher Nasser, Nour Karra, Oren Giladi
The Institute for Drug Research of the School of Pharmacy,
Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Despite significant progress in cancer research and wide availability of potent drugs, treatment efficacy is still hindered by the lack of pharmaco-selectivity to diseased cells, indiscriminate drug toxicities and poor patient compliance. Nanocarriers hold great potential in cancer treatment as they can selectively deliver high drug payloads to tumors by passive or active targeting processes while minimizing systemic exposure and adverse-effects. Active targeted polymeric nanoparticles are considered to be the most promising drug-loaded nanocarriers that can be conjugated to various ligands able to deliver the drug to the specific desired pathological tissue. This targeting strategy is based on the molecular recognition of tumor bio-markers which are over-expressed on cancer cells via specific vector molecules conjugated to the surface of the drug carrier. These vector molecules dictate the carrier's biological affinity to the desired site of action. The actual presentation will mainly focus on the potential for improving cancer treatment using twopaclitaxel palmitate-loaded nanoparticles (NPs) conjugated either to cetuximab or trastuzumab by means of oleyl cysteineamide, a novel synthesized amphiphilic linker molecule, anchored at the interface of NPs producing thiol surface activated NPs. Overall results, yielded by the cetuximab-conjugated NPs, indicate the potential of this promising platform for improved lung cancer treatment. In thetrastuzumab NP project, a radioisotope chelated using a DOTA derivative was also conjugated to the NP by the same linker molecule. This platform can make use of either a diagnostic or therapeutic radioisotope and represents an opportunity to further improve cancer treatment.

Keywords: cancer, nanoparticles, targeting, cetuximab

STUDYING DESIGN PRINCIPLES OF BISPECIFIC ANTIBODIES

Lilach Vaks[1], Rahely Hakim[1,2], **Itai Benhar**[1]
[1]*Molecular Microbiology and Biotechnology, Tel Aviv University, Israel*
[2]*R&D, Fusimab, 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. Therapeutic mAbs are mostly of the IgG format, and 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 not with a heavy chain identical to itself. 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 first criterion was described in 1996 by a group of investigators from Genentech, termed "knobs into holes" (KIH). Until recently, no solutions for the second criterion were suggested. We present a solution for the second criterion; an engineered disulfide bond between the antibodies' variable domains, an approach termed disulfide stabilization, replacing the natural disulfide bond between the CH1 and CL domains. By combining KIH for the heavy chains and disulfide stabilization for light chains we efficiently produced several BsAbs using our recently-described system for expression of IgGs in bacteria ("Inclonals" technology) in in a mammalian cells expression system. In the presentation examples will be provided for the evaluation of some of these BsAbs and future directions of the study will be discussed.

Keywords: Therapeutic monoclonal antibodies, Bispecific antibodies, disulfide stabilization

CELL DERIVED VESICLES: TURNING NATURAL TARGETING TO SMART DRUG DELIVERY SYSTEMS

Marcelle Machluf
Department of Biotechnology and Food Engineering, Technion, Israel

Homing therapeutics using drug delivery systems to different pathological states need still to fulfill the 'magic-bullet' requirements, providing versatile site-specific targeting platforms to multiple disorders, in a clinically relevant design. In our lab we aim to target disorders such as HIV, cancer and inflammatory conditions of the heart using novel drug-delivery systems based on vesicles derived from the cytoplasmatic membranes of cells. In particular we emphasize the use of stem cells known to target, either naturally or when engineered, these pathological conditions, mostly through membrane interactions. These easily prepared vesicles resemble nano-liposomes in structure; however they are already armed with naturally or engineered unique ligands, which allow their in *vitro* and predominantly *in vivo* targeting to the area of interest while being rapidly cleared from non-targeted organs. Most importantly such vesicle can be designed to be non immunogenic paving the way for future clinical applications.

DELIVERING INSTRUCTIVE CUES AND IMAGING BIOMOLECULES TO 3D ENGINEERED TISSUES

Assaf Shapira, Michal Shuman, Neta Soffer-Tsur, **Tal Dvir**
Department of Biotechnology, Faculty of Life Sciences, Tel Aviv University, Israel

Drug delivery systems have allowed the sustained release of bioactive molecules to engineered tissues, with marked effects on tissue function. In this talk I will present new approaches in the field of tissue engineering relying on natural control release systems supplying factors to engineered tissues. In addition, technologies allowing distinction between various cell types in 3D co-cultures, enabling real time monitoring of cell and tissue fate will be presented.

Keywords: 3D scaffolds, Tissue engineering, Growth factors

CHOP, DOWNSTREAM TO ENDOPLASMIC RETICULUM STRESS, IS A CRITICAL REGULATOR OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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[2]*Liver Unit, Sourasky Medical Center, Israel*

The liver is a major site of drug metabolism and elimination and as such is susceptible to drug toxicity. Drug induced liver injury (DILI) is a leading cause of acute liver injury, of which acetaminophen (APAP) is the most frequent cause. APAP toxicity is initiated by the formation of its toxic metabolite NAPQI. However, downstream mechanisms underlying APAP-induced cell death are still unclear. Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) have recently emerged as major regulators of metabolic homeostasis. UPR regulation of the transcription repressor CHOP is known to promote cell death. We analyzed the role of the UPR and CHOP in mediating APAP hepatotoxicity.

Methods: A toxic dose of APAP was orally administered to wild type and CHOP knockout mice.

Results: ER stress and the UPR were activated 12h following APAP administration. CHOP was upregulated only following the induction of the UPR. CHOP knockout mice were protected from APAP induced damage and exhibited decreased liver damage and necrosis and increased survival. APAP metabolism in CHOP knockout mice was normal but glutathione depletion and oxidative stress were decreased. Remarkably, CHOP KO but not wt mice exhibited hepatocyte proliferation at sites of necrosis.

Conclusions: We suggest that APAP activates the UPR leading to up-regulation of CHOP which curtails regeneration following liver damage. Our findings raise a potential new point of view on the role of UPR in APAP toxicity. In this case, through the activation of CHOP, UPR interferes with liver regeneration.

Keywords: Drug induced liver injury

NEXT STEP IN DRUG TARGETING: INSIDE CELLS AND TO INDIVIDUAL ORGANELLES

Vladimir P. Torchilin

Director, Center for Pharmaceutical Biotechnology and Nanomedicine; Director, Center for Translational Cancer Nanomedicine, Northeastern University, USA

Ideal drug carrier is expected: (a) to accumulate in required organ or tissue, then (b) penetrate inside target cells delivering there its load (drug or DNA), and then (c) target individual organelles. Organ or tissue accumulation could be achieved by the passive targeting via the enhanced permeability and retention effect or by antibody-mediated active targeting, while the intracellular delivery could be mediated by certain internalizable ligands or by cell-penetrating ligands, while organelle targeting requires additional special arrangements.

This requires the development of multifunctional drug delivery systems (long-circulating, specifically targeted, and capable of cell penetration and organelle targeting) built in such a way that during the first phase of delivery, cell-penetrating function is "switched off". Upon accumulating in the target, protecting polymer or antibody attached to the surface of pharmaceutical carrier via the stimuli-sensitive bond should detach under the action of local pathological conditions and switch on the function allowing for the subsequent delivery of the carrier and its cargo inside cells.

Intracellular drug delivery with subsequent organelle targeting opens new opportunities in overcoming problems associated with multiple pathologies including lysosomal storage diseases and multidrug resistance (MDR) tumors. Delivery of deficient enzymes for the treatment of lysosomal diseases evidently requires specific targeting of lysosomes, while facilitating apoptotic cell death in MDR tumor would require targeting of mitochondria or lysosomes. Examples of specific targeting of lysosomes and mitochondria in cells illustrate the benefits of this new approach.

Keywords: drug delivery, lysosomal storage diseases, multidrug resistance (MDR) tumors

PEGYLATED NANO-LIPOSOMES LOADED WITH AMPHIPATHIC WEAK ACID GLUCOCORTICOSTEROIDS FOR SUCCESSFUL SYSTEMIC TREATMENT OF ANIMAL MODELS FOR RHEUMATOID ARTHRITIS, MULTIPLE SCLEROSIS, CEREBRAL MALARIA AND CANCER

Yuval Avnir[1], Keren Turjeman[1], Yaakov Naparstek[2], Rina Ulmansky[2], Simcha Evenchen[1], Judith Waknine[1], Jacob Golenser[1], **Yechezkel Barenholz**[1]

[1]*IMRIC, Hebrew University-Hadassah Medical School, Israel*
[2]*Department of Medicine, Hadassah University Hospital, Israel*

Glucocorticosteroids (GC) are highly efficacious drugs in a wide range of diseases with an inflammatory component including RA, MS, CM and cancer (where they may have dual effects as anti-inflammatory and anticancer drugs). In the acute phase of these diseases steroids have to be given systemically at high doses. However, such systemic administration is limited by the severe adverse effects and the inferior pharmacokinetics (PK) and biodistribution (BD). This explains why a liposomal steroid formulation was an extensive development target since the use of liposomes as drug carriers was initiated (1971). However, so far, due to misunderstandings about the relevance of the physicochemical properties of most existing steroids, there is no such formulation in clinical use. We tried to think "out of the box" and to overcome the lack of suitability of most existing steroids by matching the selection of steroid pro-drugs with the right properties of the liposomes. The use of amphipathic weak acid GC pro-drugs enables achieving a stable remote loading at very high drug to lipid mole ratio (sufficient to get therapeutic efficacy) into pegylated nano-liposomes. These liposomes have a long circulation time that, due to the method of loading and the specific liposomes' lipid composition, retain enough drug during circulation so that when the nano-liposomes get selectively into the inflamed and/or cancerous tissue (due to the selective porosity of the blood vessels) there is a slow zero drug release there. Thus, superior therapeutic efficacy and therapeutic index over the free (non-liposomal) drug are achieved. In this presentation we will demonstrate the importance of using the optimized physicochemical variables of the liposomes and the drug to the successful development and the excellent in vivo performance of the liposomal steroid nano-drug in several animal models such as for RA, MS, CM and cancer. We will also show and explain the in vitro / in vivo correlation (IVIVC) of our preferred nano-drug formulations.

Keywords: Glucocorticosteroids, liposome, nano-drug

Acknowledgments: The Barenholz Fund, the ISF (partial support to the CM project).

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A NOVEL FORMULATION OF LIPOSOME CO-ENCAPSULATED ALENDRONATE AND DOXORUBICIN FOR CANCER THERAPY

Alberto Gabizon
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Nitrogen containing bisphosphonates (NBPs) are potent inhibitors of farnesyl pirophosphate synthase, the major enzyme of the mevalonate pathway with direct and indirect antitumor effects in various types of cancer, as well as important immunological effects.NBPs are currently under extensive evaluation for use in combination therapies for various anti-tumor applications. Rapid elimination from plasma by renal clearance, minimal tissue penetration (apart from bone), and poor cellular permeability substantially undermines their anti-tumor potential. To improve their pharmacokinetic profile and to enable co-delivery with chemotherapy, we developed a pegylated liposome formulation of a dissociable salt of the NBP, alendronate (Alend), with doxorubicin (Dox), a commonly used chemotherapeutic agent (PLAD).

Liposome encapsulated alendronate was used to generate a gradient to drive Dox into liposomes, forming a salt that holds these drugs in the liposome water phase. Thus the resulting formulation contains 2 active principles (Alend and Dox) with completely different mechanisms of action (known to be synergistic in some models) and non-overlapping toxicities, in which one agent is the driving force for encapsulation of the other by remote loading. In addition, a folate-receptor targeted PLAD formulation was designed with long circulation time, allowing both passive and active tumor targeting using a folate ligand to facilitate intracellular delivery into tumor cells that over-express the folate receptor. *In vitro* and *in vivo* experiments done with PLAD in non-targeted form and in folate-receptor targeted form will be presented. This new formulation appears to be very stable with similar pharmacokinetics of Alend and Dox, and a circulation half-life comparable to that of pegylated liposomal doxorubicin sulfate (known commercially as DOXIL). Cytotoxicity tests in a number of human carcinoma cells lines demonstrated reduced IC50 values for the co-encapsulated formulations when compared to DOXIL, with further enhancement by folate-targeting in folate receptor expressing cell lines, suggesting synergism for the co-delivery of Alend and Dox. In vivo, improved efficacy of PLAD over DOXIL was found in the M109R mouse carcinoma model. An update on the pharmacology and development of this formulation will be presented.

Keywords: Alendronate, Doxorubicin, liposome, cancer

GAGOMERS: NOVEL SAFE PARTICLE CLUSTERS FOR SYSTEMIC DELIVERY OF RNAI PAYLOADS

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CD44, a well-documented cell surface receptor, is involved in cell proliferation, migration, ignaling, adhesion, differentiation and angiogenesis, which are important properties for normal and cancerous cell function. We recently developed particle clusters coated with hyaluronan, CD44 ligand, (termed gagomers; GAGs), and showed that they can deliver paclitaxel, and separately Mitomycin C (MMC) directly into CD44-overexpressing tumors in mouse tumor models.

We also showed that GAGs can bind to primary head and neck cancers (HNC) and deliver MMC to these cells in an efficacious manner, but not to normal cells taken from the same patient. Here we show that GAGs can entrap high amounts of RNAi payloads and deliver them directly into tumor-bearing mice in a selective manner without any bystander delivery. Next, we show that RNAi formulated in GAGs do not elevate cytokines, or induce interferon response or activate complement in human peripheral blood mononuclear cells, mice and rats. In addition, histology sections do not reveal any pathological changes in organs of the mononuclear phagocytic system (MPS) such as the liver, spleen, and lungs. In addition, no liver enzyme release or changes in metabolic parameters are observed. These results demonstrate the selective recognition of GAGs towards cancerous cells with an exquisite capability to deliver RNAi payloads and induce robust therapeutic gene silencing together with excellent safety profile. These credentials position GAGs as an attractive alternative to cationic formulations, which might be applicable in many types of cancer and inflammatory disorders.

IMPLEMENTATION OF QUALITY BY DESIGN- A NEW APPROACH TO DRUG DEVELOPMENT

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An overview of Quality by Design approach to drug development will be presented, QbD elements discussed, an update given on current status of applications, and reference made to controlled drug delivery products.

ACUTE AND CHRONIC INFLAMMATIONS TREATED BY STEROID, NSAID AND ANTIOXIDANT DRUGS ENCAPSULATED ALONE OR IN COMBINATION IN BIOMATERIAL-BASED BIOADHESIVE CARRIERS

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To increase efficacies and reduce severe adverse effects in treatments of acute and chronic inflammation, we formulated dexamethasone (Dex, a steroid), diclofenac (a NSAID) and N-acetyl-Cystein (NAC, an antioxidant), in collagomers, hyaluronan bioadhesive (HA-BAL) and collagen bioadhesive (COL-BAL) liposomes.

Exposure to toxic industrial chemicals (TIC) such as HCl and NH_3causes respiratory damage and acute lung inflammation. HA-BAL encapsulating NAC and Dex (each alone and in combination) were successfully formulated in the liposomes and aerosolized with no material losses and no changes in formulation properties. The liposomal formulations outperformed the free drugs in protecting lung cells from TIC damage. Modeled by the ACI device, the liposomal aerosols have the ability to reach deep into human lungs. Protocols for animal model studies, recently launched, will be discussed.

Diclofenac and Dex (each alone and in combination) were formulated in HA-BAL, COL-BAL and collagomers, for the treatment of Osteoarthritis. High encapsulation efficiencies and slow-release performances were obtained, and retention of therapeutic activity for all carrier systems was verified in cell cultures by their impact on activity and expression of the COX enzymes. Using live-animal MRI, a single intra-articular injection of each of the tested drug-carrier formulations sufficed to reduce knee joint inflammation in OA rats over a time span of 17 days. The best performing system was the drug combination in HA-BAL - inflammation volume down to 12.9% from initial.

Using drug-carrier formulations and administration routes befitting the therapeutic indication has high potential to improve clinical outcomes in acute and chronic inflammations.

Keywords: inflammation, liposomes, bioadhesion, inhalation, osteoarthritis

TARGETED THERAPY = PERSONALIZED MEDICINE?

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Personalized medicine has become the "white-knight" that will come to the rescue of the ailing health-care system. It will solve the "innovation-draught" problem, will improve the quality of medicine, reduce treatment side-effects, and solve the financial crisis of the health-care system. Targeted therapy is considered to be one of the pillars of the personalized medicine vision as it can be applied exclusively to patients who carry the target, thus improving therapy outcome and eliminating unnecessary side effects and costs. Furthermore targeted therapy associated with appropriate biological markers should improve dramatically R & D efficiency. However, close examination will show that in the real world these premises should be questioned. In those cases where there is a clearly identified target (e.g. antibodies such as Herceptin against HER 2, or imatinib against bcr-able mutations) the use of the targeted therapies are not personalized to accommodate patient-specific characteristics. This is clearly reflected in the prescribing information of such drugs. In fact healthcare bodies and reimbursing bodies enforce strict clinical practice guidelines which by definition are not personalized. Furthermore according to recent survey conducted among industry R & D leaders only about 10% of future therapies will be combined with biomarkers, and in fact it is believed that the incorporation of biomarkers will increase rather then decrease development costs. Furthermore physicians, healthcare managers and health insurance bodies are reluctant to use and reimburse the routine use of biomarker testing. Beyond these mundane issues, recent research creates doubts about the whole concept of personalized medicine based on targeted therapy as: "….intratumor heterogeneity can lead to underestimation of the tumor genomic landscape portrayed from single tumor-biopsy samples and may present major challenges to personalized-medicine".

Keywords: Personalized medicine, Targeted therapy, R & D

IP STRATEGY FOR DRUG DEVELOPMENT

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IP Strategy in general and IP strategy for drug development in particular is a very complex issue. After a brief introduction to the world of IP, the lecture will focus on the major IP issues associated with drug development, including,*inter alia*, the generation of an IP portfolio aimed at New Chemical Entity (NCE), NCE Analogs, NCE Optimization, Mechanism of action, Drug candidate, Formulation (e.g., controlled release), Manufacturing, Clinical trial (First indication …, Regimen , Dosing, Inclusion/Exclusion Criteria), Co-formulation, Up-scaling production, Isomers, Crystalline forms, Pro-drugs and Salts.

Keywords: Drug development, strategy, patents

BonyPid™: A LIPID-AND-POLYMER-BASED NOVAL DRUG DELIVERY SYSTEM: PHYSICOCHEMICAL ASPECTS AND THERAPY

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Bacterial infection of bone may result in bone destruction which is difficult to cure due to poor accessibility to bone of systemically-administrated antibiotic and poor performance of currently available local antibacterial treatments. PolyPid Ltd developed a novel local drug delivery system based on self-assembly of pharmaceutically approved lipids and polymers that encapsulate doxycycline (Doxy). The formulation is self-assembled lipid matrix via the interaction of the lipids and biocompatible - biodegradable polymer. The entrapped Doxy is located within the anhydrous environment and therefore fully protected from long-term water-exposure-related degradation. The fine coating of the bone filler by this formulation (BonyPid™) is capable of releasing active drug at zero-order kinetics for a predetermined period of up to 30 days.

The wide angle X-ray analyses (WAXS) of BonyPid™ samples are suggesting that the polymer and lipid coating is a highly organized nano-substructure. The principle lipid in BonyPid™ formulation is isphosphatidylcholine. It was found that the length of the acyl chains can significantly alter the release rate of Doxy during the zero-order release phase.

The anti-infection activity of BonyPid™ was tested in the rabbit tibia model contaminated with $5x10^5$ *S. aureus.* Both acute and chronic infection models were tested. Only BonyPid™ treatment demonstrated a statistically significant reduced bone absorption over the infected group ($P<0.04$) and significantly lower bacterial bone concentration ($p>0.05$).In addition, BonyPid™ did not reducebone hilling as compared to non-coated bone-filler.

Clinical evaluation is proposed for testing the efficacy and toxicity of BonyPid™.

Keywords: Bone infection, Antibiotic, Zero-order kinetics

NANO-SILENCING FROM TRYPANOSOMES TO MAN

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The challenge in developing siRNA/microRNAs into drugs lies on their ability to enter cells. Despite many existing studies involving nanoparticles (NPs) for such a delivery, basic knowledge is lacking on how NPs enter cells, release from the endocytic pathway and interact with the silencing machinery. In this study, we explored the mode of entry and the potential for gene silencing of two types of NPs building on a core of silica (SiO_2) or CAN-maghemite (CAN-Fe_2O_3) functionalized with a thin adlayer of polyethyleneimine (PEI).
The NP entry to various cancer cells and trypanosome parasites as the causative agent of devastating diseases was investigated. The trypanosome surface is retractile to the entry of external materials due to a tight tubulin layer present underneath the plasma membrane enabling entry of size-limited particles (< 80 nm) via its flagellar pocket. Both silica-based and the CAN-maghemite particles enter all the cancer cells tested but only the 50 nm-sized CAN-maghemite ones enter *Trypanosoma brucei*. Different inhibitors were used to assess their mode of cellular entry. Data show that the mode of NP entry differs with the surface composition of the particle, suggesting that the entry of siRNA-covered NPs occurs via Caveole-mediated phagocytosis utilizing actins for entry. Efficient silencing was obtained for both **mRNA** and **mir-21** using both NP types. Studies are in progress to develop such tools for gene silencing in trypanosomes as potential drugs against parasite-mediated devastating diseases.

Keywords: Nanoparticle engineering, siRNA gene silencing, microRNA gene silencing

TARGETING AND LYSIS OF BACTERIA, FUNGI AND CANCER BY HOST-DEFENSE-LIKE MEMBRANE ACTIVE PEPTIDES AND NANO-PARTICLE LIPOPEPTIDES

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Short peptides, lipopeptides, and protein toxins are produced by all forms of life and they utilize various mechanisms to combat pathogens invasion. Cationic host defense antimicrobial peptides (AMPs) are the largest group within this family. These peptides are characterized by a net positive charge and a threshold hydrophobicity, enabling them to target, bind, and lyse preferentially negatively charged membranes, which are enriched in the outer leaflet of bacteria, fungi, and slightly in cancer cells. Biochemical, biophysical and biological studies suggest that membrane lysis occurs after a threshold concentration has been reached. We named this process a "carpet" mechanism. Based on the "carpet" mechanism, we designed novel families of diastereomeric (containing D- and L-amino acids) peptides and nano-structured ultra-short lipopeptides. These compounds show cell specific killing activity against a variety of target cells. They have promising properties which make them attractive templates for the development of future antibiotics with new modes of action, to which it will be difficult for pathogens to develop resistance.

CRYSTALLIZATION AND RELEASE OF RAPAMYCIN FROM METALLIC STENTS

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Drug-eluting stents (DES) have become an accepted technology in intravascular intervention. Manufacturing methodologies of DES are based mainly on mechanical processes such as spray and dip coating of a polymer solution containing a drug. In the present work, several methods for applying rapamycin onto stents were developed, including electrocoating of pyrrole derivatives and direct crystallization of the drug onto metal surfaces.

Pyrrole and aromatic diazonium derivatives have been synthesized and applied onto stents by electropolymerization. These surface modifications allowed absorption of rapamycin or paclitaxel onto the stent surface that provided an extended release of the loaded drug. Rapamycin was crystallized onto the metallic surface in two steps, seeding with drug particles and crystal growth by immersing the stent into a solution of the drug. In vitro release was determined in phosphate buffer solution pH7.4 at 37^{o}C.

Nanolayers onto metallic stents have been prepared by electrografting on acrylic monomers onto stainless steel stents following radical polymerization to form an organic thin layer which allowed improved adhesion of a drug loaded secondary layer which releases the drug for weeks without detachment of the coating under physiological conditions. Rapamycin nanocrystals have been seeded onto stents which served as nucleation spots for the farther crystal loading onto stents to form a non-polymer drug eluting stent releasing the drug for weeks. DES can be achieved by either electrocoating of stent surface or by crystallization of the drug onto the stent surface.

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Keywords: rapamycin, DES, crystallization

SELF-ASSEMBLY OF BIOMOLECULAR NECKLACES

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Nature utilizes simple building blocks such as nucleic acids, phospholipids and amino acids to create complex functional structures by the process of molecular self-assembly. In an effort to mimic this process *in vitro,* numerous studies have demonstrated the ability of DNA molecules, peptides and lipids to assemble into ordered structures. The potential of these structures in a wide range of nanotechnological and biotechnological applications is immense. Peptides, specifically, hold a great promise as biomolecular building blocks since they present diversity, easy to synthesize in large scale, and can be easily modified with biological and chemical groups. The ability to spontaneously form peptide-based structures with the degree of complexity found in nature is, however, still a challenge. The lecture will present a new approach for the spontaneous formation of biomolecular architectures via the self-assembly of peptides. Using this strategy we discovered a unique structure that combines elongated elements and spherical assemblies. The new structure resembles in its morphology to beaded strings as the spherical structures seem to be threaded on the elongated ones. We, therefore, termed these assemblies "biomolecular necklaces". This newly discovered peptide architecture can potentially serve as a scaffold in bioengineering applications or as a carrier for drug delivery.

Keywords: Self-assembly, peptide, molecular recognition, necklaces

REAL-TIME DIAGNOSTICS OF GI MALIGNANCY BY DRUG DELIVERY TOOLS

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Early detection is imperative for the prevention and efficient treatment of gastrointestinal (GI) malignancy. This could be attained by real time diagnosis, joining improved imaging and pharmaceutical approaches for the detection of biomarker molecules either in the lumen or the mucosa of the alimentary canal. We have exploited a variety of strategies to tackle this challenge. Employing a1- antitrypsin precursor (A1AT) as a secreted biomarker model of gastric carcinoma, we developed a platform with immunoassay capabilities, comprising a sensing and detecting compartments. It was made of a microarray-type support grafted with trypsin as a capturing moiety and a detecting compartment made of near infrared (NIR) fluorescently labeled nanoparticles conjugated to A1AT-specific antibodies (1).

For the early detection of colon polyps we prepared a fluorescently labeled (NIR range) cationized polyacrylamide aimed at targeting the overexpressed sialic acid in colonic malignant cells and tissues. The specific attachment of the polymer was tested in a series of cancer cells and found to be CRC staging dependent. The optimal polymeric product was tested, successfully, in gut sac preparations of the dimethylhydrazine induced rat model. To increase the polymer's targeting capabilities, a FITC labeled recognition peptide (EPPT1) that targets the transmembrane glycoprotein underglycosylated MUC-1 was conjugated to the polymeric backbone and tested in HT-29 and LS-147T cells, followed by an *in vivo* examination in an orthotopic mouse model. Only the lowest EPPT1 molar ratio in the dually recognition polymer increased the polymer binding to the cells, probably due to quenching phenomena and steric hindrance (2).

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Keywords: *In vivo* diagnostics, GI malignancy, targeting

QUADRUGNOSTIC NANOPARTICLES FOR CANCER THERAPY

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Anticancer drug resistance frequently emerges, posing major obstacles towards curative therapy of various malignancies. Certain mechanisms of chemoresistance are mediated by ATP-driven multidrug resistance (MDR) efflux transporters of the ATP-binding cassette (ABC) superfamily. Recently, nanoparticle (NP)-based therapeutic systems have been developed that were rationally designed to overcome drug resistance by neutralizing, evading or exploiting various drug efflux pumps and other resistance mechanisms. Here we devised a novel nanomedicine platform which we term a "quadrugnostic nanoparticle"[1] (QNP), that is envisioned to be the next generation platform for simultaneous cancer diagnostics and therapeutics (theranostics). These QNPs will harbor in the same vehicle, **four** synergistic components including: (1) a selective targeting moiety, (2) a diagnostic imaging aid for the localization of the malignant tumor and its distant metastases, (3) a cytotoxic, small molecule drug(s) and (4) a chemosensitizer(s) aimed at neutralizing a well-defined drug resistance mechanism. We present the first QNP prototype based on self-assembling conjugates of a hydrophobic molecule conjugated to a hydrophilic polymer- hyaluronic acid, which also serves as an active-targeting moiety, targeting cancer cells overexpressing CD44 (e.g. ovarian cancer). In addition, this QNP will harbor a cytotoxic drug, and a chemosensitizer, to overcome MDR. The QNP will be also decorated by an imaging-aid. Preliminary results showed that the conjugates formed self-assembled NPs and bound hydrophobic cytotoxic drugs with high affinity. The following steps will include completing the construction of the quadrugnostic nanoparticles, and evaluating their cytotoxic activity against MDR cancer cells *in vitro* and *in vivo.*

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Keywords*:* Cancer; Chemotherapy; Multi-drug resistance (MDR)*; Quadrugnostic; Nanomedicine*

LIPOSOMAL BISPHOSPHONATE MECHANISM OF ACTION IN INHIBITING RESTENOSIS: EFFECT ON MONOCYTES SUBPOPULATIONS.

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Encapsulating a BP in a particulate delivery system, such as nano-liposomes (LIP-BPs), enables specific targeting and depletion of circulating monocytes and macrophages of the mononuclear phagocytic system (MPS). Partial and transient systemic depletion of monocytes and acrophages by LIP-BPs reduces neointimal hyperplasia and restenosis in animal models. Furthermore, a single IV injection of liposomal alendronate is now under phase II clinical trials for preventing in-stent restenosis. Recent studies have shown that monocytes are a heterogeneous population of cells. Based on several criteria (including size, granularity, expression of receptors to chemokines and activity) the monocytes can be divided into sub-populations of "classical" (CM) and "non-classical" (NCM). These sub-populations are characterized in human ($CD14^{hi}CD16^{-}$ and $CD14^{hi}CD16^{+}$ respectively), in mice ($Ly6C^{hi}$ and $Ly6C^{low}$ respectively) and in rat ($CD43^{low}$ and $CD43^{hi}$ respectively). "Classical" monocytes exhibit pro-inflammatory properties, while "non-classical" monocytes exhibit anti-inflammatory properties. We hypothesized that the effect of LIP-BPs on monocytes subpopulations could elucidate the pathophysiology of restenosis and the drug's mechanism of action. In this work we investigated the effects of liposomal alendronate (lipALN) on blood monocytes subpopulations in the rat balloon-injury model of restenosis. At the time of the injury animals were treated with a single injection of high-dose lipALN, low-dose lipALN or saline. CM and NCM were determined by FACS as $ED1^{+}CD43^{-}$ and $ED1^{+}CD43^{+}$, respectively. High-dose lipALN changed the ratio of CM:NCM in favor of NCM at 24h, depleted monocytes after 48h and elevated CM at 7d, while low-dose lipALN treatment resulted inCM:NCM changed ratio in favor of NCM, persisted for 7d without monocytes depletion (vs. saline). It is concluded that the ratio of CM:NCM followed by depletion of monocytes by high-dose lipALN is associated with the therapeutic effect exhibited in restenotic rats.

Keywords: monocytes subpopulations, liposomal bisphosphonates

RELEASE OF ANTIBODIES FROM GOLD NANOSPHERES DURING SHORT-PULSE LASER PHOTOTHERAPY

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A method for nano-manipulations of malignant cells have been recently developed in our laboratory, using gold nanospheres conjugated to specific antibodies and illuminated by ultrashort laser pulses of wavelength tuned to the particles' plasmonic resonance. While the targeting antibodies on the nanoparticles are used primarily for specific attachment of the nano-conjugates to the cancer cells, they can also be used for triggering the complement-dependent cytotoxicity system, once optically released from the nanoparticles. We have found that complement-dependent cytotoxicity had become significant following pulse illumination, indicating the release of the antibody molecules from the gold particles. The talk will review the possible mechanisms which may have led to this effect and discuss the overall context of this technology.

Keywords: gold nanoparticles, ultrashort pulses, cancer cells

GASTRIC RETENTIVE ORAL DOSAGE FORMS – WHEN REALITY MET THEORY

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Gastric retentive dosage forms have been developed in the past years to provide a solution for control release therapy for drugs with narrow intestine absorption window. The major investigated concepts are based on (1) low or high density tablets that float or sink in the gastric content, respectively, such that the transition to the small intestine is prevented; (2) dosage forms that contains bioadhesion compounds which adhere the dosage form to gastric tissue, such that passage through pylorus sphincter is delayed; (3) swelled dosage forms such that it becomes too large to pass through the pyloric.

Currently, the polymeric swelling monolithic systems are the most prominent marketed forms. In this presentation, we will describe some of our experience which accumulated during the development of generic versions to gastric swelling dosage forms, the effect of tablet size on gastric retention, and a novel concept of non-swelling gastric retentive dosage forms.

Keywords: Gastric Retentive, Swelling, Oral Dosage Forms

MOLECULAR TREES: FROM THE TEST TUBE TOWARDS BIOMEDICAL APPLICATIONS

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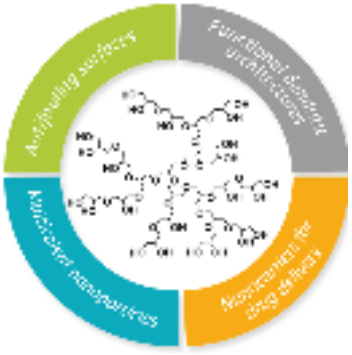
The diversified pathophysiological scenarios for many different disease conditions continually call for innovative and novel therapeutic approaches. In line with conventional treatment strategies, the application of nanotechnology in medicine and pharmaceuticals is a rapidly moving field that is gaining fast acceptance and recognition as an independent area of research and scientific endeavor.[1,2,3] The combination of a high density of endgroups and a compact well defined molecule structure makes dendritic architectures attractive for biomedical applications. The synergy between their multivalency and size in nanoscale provides a range of options for chemical "smartness" along their molecular scaffold to achieve environment sensitive modalities.

Due to their low degree of molecular weight dispersity, flexible design, and biocompatible nature, dendritic polyglycerols (PGs) have a broad range of potential applications in medicine and pharmacology.[4,5]



Dendritic polyglycerol architectures have already been demonstrated to be useful in therapeutic approaches related to multivalency because of the synergy between the nano-sized dimensions combined with the high density of functional groups.[6,7] A challenging approach to the application of multivalent interactions is the mimicry of functional biomacromolecules with therapeutic relevance.

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Keywords: nanotechnology, dendritic polyglycerols, molecular trees

GOLD NANOPARTICLES AS COMPUTED TOMOGRAPHY IMAGING CONTRAST AGENTS

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Computed Tomography (CT) plays a critical role in overall cancer management: in diagnostics, staging and radiation therapy treatment planning. Recently, advanced radiation and surgical techniques have been developed, such asIntensity Modulated Radiation Therapy (IMRT) and focused robotic and endoscopic surgery that enable tumor targeting with sub-mm spatial resolution. However, these techniques are limited by the low specificity and sub-cm spatial resolution of tumor visualization and delineation provided by the CT. our recent progress in developing specifically targeted nanoparticle-based CT contrast agents for molecular imaging of cancer will be described, and in vivo applications for colorectal, head and neck, and brain cancer will be presented. This technique, which expands the role of CT beyond its present structural imaging capabilities and provides it with functional and molecular-based imaging capacities as well, is expected to improve treatment through early detection of millimeter-sized tumors using clinical CT.

LIPOSOME IMAGING AGENTS FOR PERSONALIZING TREATMENT WITH NANOCARRIERS

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Nanoparticles are well established as effective drug delivery systems and have potential in biomedical imaging as a diagnostic tool. We have recently developed a highly efficient method for utilizing liposomes as agents in positron emission tomography (PET) imaging giving high resolution images and allowing direct quantification of liposome tissue distribution and blood clearance. Our approach is based on remote loadingof a copper-radionuclide (^{64}Cu) intopreformed liposomes and copper entrapment by an encapsulated copper-chelator. We show thatthe^{64}Cu-liposomes provide quantitative*in vivo*imaging in canines with spontaneous tumors using PET. Seven canines with spontaneous tumors were included in the studywhere the main focus was to evaluate the EPR effect in large animals with spontaneous tumors and the performance of the developed liposome imaging agent.None of the included dogs displayed any anaphylactic, toxic or adverse reactions.Liposome circulating half-life ranged from 24.2 hours to 54.2 hours, with a mean half-life of 35.0 ± 4.24 hours. The study showed that the EPR effect assures substantial tumor accumulation in some but not all spontaneous tumors in canines.The included carcinomas displayed higher mean and maximum uptake levels of liposomes relative to the included sarcomas.The^{64}Cu-liposomes have potential as a diagnostic tracer in cancer diagnostics. We envision that the^{64}Cu-liposomes will be an important tool for evaluating liposome performance in future and may become an important tool in selection of cancer patients for nanoparticle based chemotherapy.

Keywords: Cancer Imaging, Liposome, Diagnostics, Theranostics

PEPTIDE NUCLEIC ACIDS (PNAs) IN CANCER DIAGNOSIS

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Peptide nucleic acids (PNA) are DNA analogs that avidly bind to DNA and RNA and are highly resistant to degradation in a biological medium. We have exploited these molecules for two purposes: a) detection of endogenous mRNA in living cancer cells at a single base pair resolution and b) detection of a non-coding RNA that was found to be highly expressed in a variety of cancer cells. We have targeted the kRAS mRNA with PNAs modified with thiazole orange (TO) as a base surrogate (1) and shown that such PNAs light up in living (un-fixed) cancer cells upon hybridization to an RNA transcript with a specific mutation in kRAS (PANC-1 cancer cells). Importantly, the same PNA showed no detectable fluorescence in cells (HT29) that have a single mis-match to the designed PNA. (2) In addition, we have used cell permeable PNAs as molecular beacons (MBs) for the detection of a recently identified cancer biomarker (3), namely, colon cancer associated transcript-1 (CCAT-1). This non-coding RNA was detected by PNAs both in living cells expressing CCAT-1 and in human biopsies taken from CRC patients by fluorescence in-situ hybridization (FISH). These studies highlight the potential of PNA molecules in screening RNAs as bio-markers for the early detection of cancer.

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Keywords: molecular beacon, peptide nucleic acids, non-coding RNA

QUENCHED ACTIVITY BASED PROBES FOR SIMULTANEOUS DETECTION AND THERAPY OF CANCER

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The cysteine cathepsins proteases are overexpressed in various cancers and are thought to be involved in tumor formation, tumor growth, tumor invasion, and metastasis. Development of sensitive and reliable probes is the bottleneck of the molecular imaging field. Here we describe the development of a technology that can be used for simultaneous detection and treatment of cancer over expressing cathepsins. In addition to providing a fluorescent signal upon target modification, the reporter serves as a photosensitizer that is then photo-activated to generate reactive oxygen species (ROS) leading to cell death of the cancerous tissue. Our designed agents consist of a quenched fluorescent reporter that is activated by specific covalent modification of the target cathepsin proteases found primarily in cancer cells. We present here the development of photodynamic quenched activity-based probes and non-quenched control probes. These probes were evaluated *in vitro* for quenching efficiency, cathepsin binding, cell permeability, cathepsin selectivity and importantly, cell killing capability by light activation. We are currently evaluating the quenched probes for non-invasive imaging capabilities and photodynamic therapy applications in subcutaneous cancer mouse models. This technology is attractive since it targets photosensitizers to cancer tissue by protease overexpression in addition to reducing the light toxicity of photosensitizers achieved by the quenching technology. Furthermore, the generation of a fluorescent signal in areas of high cathepsin activity in vivo is attractive for the Nano medicine field allowing for the identification of regions of high drug release from Nano-carriers.

DIRECTED EVOLUTION OF AFFINITY SCAFFOLDS FOR DRUG DELIVERY AND BIOMEDICAL DIAGNOSTICS

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Significant crosstalk exists between receptors that mediate angiogenesis, such as vascular endothelial growth factor receptor-2 (VEGFR2) and $\alpha_v\beta_3$integrin. Thus, agents that inhibit both receptors would have important therapeutic potential. Here, we used an antagonistic VEGF ligand as a molecular scaffold to engineer dual-specific proteins that bound to VEGFR2 and $\alpha_v\beta_3$integrin with antibody-like affinities and inhibited angiogenic processes *in vitro* and *in vivo*. Mutations were introduced into a single-chain VEGF (scVEGF) ligand that retained VEGFR2 binding, but prevented receptor dimerization and activation. Yeast-displayed scVEGF mutant libraries were created and screened by high-throughput flow cytometric sorting to identify several variants that bound with high affinity to both VEGFR2 and $\alpha_v\beta_3$integrin. SPR and cell binding assays showed that the dual-specific proteins can simultaneously engage both receptors. Compared to mono-specific scVEGF mutants that bind VEGFR2 or $\alpha_v\beta_3$integrin, dual-specific proteins more strongly inhibited VEGF-mediated receptor phosphorylation, capillary tube formation, and proliferation of endothelial cells cultured on Matrigel or vitronectin-coated surfaces. Moreover, dual-specificity conferred complete inhibition of VEGF-mediated blood vessel formation in Matrigel plugs *in vivo*. The dual specificity was also associated with near-complete neutralization of VEGF corneal micropocket angiogenesis in mice and with high tumor uptake, as observed using whole body near-infrared (NIR) optical imaging in glioblastoma xenograft mouse model. Instead of relying on antibody associating domains or physical linkage, this work highlights an approach to creating dual-specific proteins where additional functionality (affinity) is introduced into a protein ligand to complement its existing biological properties. These protein engineering tools allow us to evolve other molecular 'scaffolds' and generate proteins with novel binding specificities and desired properties for applications in drug and gene delivery as well as in medical diagnostics.

BENEFITS OF DIFFERENT CHARACTERIZATION TECHNIQUES FOR DRUG DELIVERY APPLICATION

Yael Shaked
Sales & Applications, Dr. Golik, Israel

There is a range of particle and molecule characterization tools which can be applied to drug development to allow better understanding of drug delivery systems and to aid rapid product development.

This short talk will provide an overview of how size, charge, molecular weight and structure can be assessed using advanced technologies as Dynamic Light Scattering (DLS), Electrophoretic Light Scattering (ELS) and Gel Permeation Chromatography (GPC/SEC).

Routinely uses within the controlled release area to improve suspension stability, maximize shelf-life, detect early stage aggregation in antibody formulations, quantify conjugation success for PEGylated proteins and measure drug carrier charge and size will be introduced and discussed.

HYBRID PEG-DENDRITIC SCAFFOLDS AS NANO-CARRIERS FOR DRUG AND GENE DELIVERY

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Dendrimers are very attractive scaffolds for the delivery of therapeutics and/or diagnostic probes, with the two major approaches of loading being: encapsulation and functionalization of the chain ends. While both approaches seem to be promising, they suffer from drawbacks due to the limited amount of cargo molecules and control over the stability of the loaded carriers. A novel delivery platform based on orthogonally functionalized hybrid dendritic-linear delivery systems that offer significant advantages in terms of loading capacity, stability and biocompatibility was developed. Evaluation of its biological activity reveals strong positive dendritic effect and promising properties. Furthermore, the interesting influence of the core architecture on the bioactivity of these hybrid macromolecules is demonstrated by their application as drug and gene delivery platforms.

THE MECHANISM OF ASSOCIATION AND TECHNOLOGICAL APPLICATIONS OF SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES

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In spite of grave clinical consequence, the mechanism of amyloid formation is not fully understood. We have suggested, based on experimental and bioinformatic analysis, that aromatic interactions may provide energetic contribution as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes. Our works on the mechanism of aromatic peptide self-assembly, 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 peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aramides. We also demonstrated the ability to use these peptide nanostructures as casting mold for the fabrication of metallic nano-wires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging [1-3].

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Keywords: Self-Assembly, Peptide Nanostructures, Nanospheres

Abstracts of posters selected for short oral presentations

Wednesday, September 5, 2012

P-1

CONTROLLED RELEASE OF RITUXIMAB FROM GOLD NANOPARTICLES FOR PHOTOTHERAPY OF MALIGNANT CELLS

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Releasing drug molecules at their targets with high spatial and temporal accuracy could aid numerous clinical applications which require low systemic damage and low side effects. Nano-carriers of drugs are an attractive solution for such task, allowing specific accumulation in tumors and gradual release of their payload. Here, we utilize gold nanospheres conjugated to Rituximab, an anti-CD20 monoclonal antibody-based drug, for carrying and releasing the drug upon irradiation of specifically tailored femtosecond laser pulses. The released anti-CD20 molecules retain their functionality and ability of triggering the complement-dependent cytotoxicity. This effect comes in addition to cell necrosis caused by the plasmonic nanometric shock waves emanating from the nanospheres and rupturing the plasma membranes. Main advantages of the presented technique include high spatial and temporal resolution, low toxicity and high repeatability and consistency due to the morphological stability of the nanospheres.

Keywords: Rituximab; Gold nanoparticles; Femtosecond pulses;

P-2

BRAIN DELIVERY OF PROTEINS USING BOLAAMPHIPHILIC NANO-SIZED VESICLES: MECHANISMS AND EFFICIENCY

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Background: The blood-brain barrier (BBB) prevents drugs' permeability into the brain and limits management of brain diseases. Specialized delivery systems are urgently required to overcome this barrier and to achieve efficient delivery of therapeutic agents to the brain. We have developed nanovesicles made of novel bolaamphiphilic compounds that can encapsulate macromolecules and can potentially deliver them into the brain.

Aims: To explore: 1) the mechanisms and efficiency of vesicle uptake by brain capillary endothelial cells *in vitro*, and 2) the ability of the novel vesicles to deliver proteins to the brain *in vivo*.

Methods: Nano-vesicles encapsulating a model protein (BSA-FITC) were prepared from bolaamphiphiles with acetylcholine head groups by film hydration followed by sonication and were characterized using analytical approaches. Uptake of the vesicles by brain microvessel endothelial cells (b.End3) was determined using FACS and confocal microscopy Tissue distribution of the encapsulated protein following systemic administration of the vesicles to mice was analyzed by fluorescence microscopy of tissue sections.

Results: Nanometric positively charged bolavesicles that efficiently encapsulated the model protein were prepared. The *in vitro* stability of bolavesicles with cleavable ACh head groups was significantly increased by pyridostigmine, suggesting that inhibition of ChE may be used to extend the circulatory survival of the vesicles *in vivo*. Internalization of the bolavesicles was inhibited at 4^{o}C and in presence of endocytosis inhibitors, indicating an active uptake mechanism by the cells. Following intravenous administration of bolavesicles to mice pretreated with pyridostigmine, significant amount of fluorescence was detected in various tissues, particularly the brain, indicating high permeability of the bolavesicles via biological barriers, including the BBB.

Conclusions: Cationic bolavesicles with ACh head groups can deliver their cargo across biological barriers, including the BBB, via endocytosis-based processes. The mechanisms of uptake and trafficking of bolaamphiphilic vesicles across the BBB are currently investigated in our laboratories.

*This study was supported by the Israel Science Foundation grant No 973/11.

P-3

HYALURONIC ACID BASED QUADRUGNOSTIC NANOPARTICLE FOR CANCER THERAPY

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Conventional chemotherapy requires sequential high doses of drug combinations which cause severe toxic side effects and exhibits limited efficacy due to the frequent emergence of drug resistance phenomena, particularly multidrug resistance (MDR). An additional challenge posed by cancer is the diagnostic localization of tumors and their distant metastases.

In the current research we developed a novel nanomedicine platform which we term a "quadrugnostic nanoparticle", that is envisioned to be the next generation platform for simultaneous cancer diagnostics and therapeutics (theranostics): These Quadrugnostic Nanoparticles (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 a hydrophilic polymer and a hydrophobic molecule. As the hydrophilic polymer, we chose hyaluronic acid, which also serves as an active-targeting moiety, targeting cancer cells overexpressing CD44 (e.g. ovarian cancer). Moreover, this QNP harbors a cytotoxic drug, and a chemosensitizer that overcome MDR based on a specific ABC drug efflux transporter. The QNP are decorated by an imaging-aid.

Preliminary results reveal that the conjugates formed self-assembled NPs and bound hydrophobic cytotoxic drugs with high affinity. The following steps will include completing the construction of the quadrugnostic nanoparticles, and evaluating their cytotoxic activity towards MDR cancer cells *in vitro* and *in vivo.*

Keywords: Cancer; Multidrug resistance (MDR)*; Chemotherapy; Quadrugnostic; Nanomedicine*

P-4

OVERCOMMING PACLITAXEL RESISTANCE WITH ANTI-ANGIOGENIC AND ANTICANCER RGD-BEARING NANOMEDICINE

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Overcoming drug resistance, emerging either on recurring cancer or metastasis, is a major challenge of cancer treatments. The combination of anti-angiogenic therapy with cytotoxic therapy offers a promising therapeutic approach. One such combination can be achieved exploiting the multvalency of polymer therapeutics. It has been shown that polymer conjugation of paclitaxel (PTX), a potent cytotoxic and anti-angiogenic drug, improved its pharmacokinetic profile. We hypothesized that actively targeting PTX via the $\alpha_v\beta_3$-integrin, presents an attractive therapeutic strategy for breast cancer. Interestingly, overexpression of $\alpha_v\beta_3$-integrin, occurring on tumor endothelial and some epithelial cells during tumor growth, invasion, and metastasis, was found to correlate with PTX-resistance of breast cancer cells.

We designed and synthesized a novel polyglutamic acid (PGA)-PTX-E-[c(RGDfK)$_2$] nano-scaled conjugate. Polymer conjugation converted PTX to a water-soluble macromolecule, which passively targeted the tumor tissue exploiting the enhanced permeability and retention (EPR) effect. The E-[c(RGDfK)$_2$] was utilized as an additional active targeting moiety to the $\alpha_v\beta_3$ integrin. PGA is enzymatically-degradable by cathepsin B, leading to PTX release. PGA-PTX-E-[c(RGDfK)$_2$] displayed a potent anti-angiogenic therapy, determined by several, well-established, *in vitro* assays. Preferential tumor accumulation of the RGD-bearing conjugate in orthotopic mammary tumors inoculated in mice, lead to enhanced antitumor efficacy and a marked decrease in toxicity compared with free PTX (Eldar-Boock *et al*., Biomaterials, 2011). The correlation between $\alpha_v\beta_3$-integrin expression on metastatic tumor cells and acquired PTX-resistance was determined *in vitro*.

Taken together, inclusion of an active targeting moiety to integrin expressing-cells has the potential to manipulate and overcome acquired drug resistance, as an anti-angiogenic and anticancer therapy.

Keywords: Polymer therapeutics, angiogenesis, overcoming drug-resistance, integrin, paclitaxel.

P-5

POLYION COMPLEXES FOR GENE DELIVERY TO DENDRITIC CELLS

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Background: Dendritic cells (DCs) are a family of specialized antigen presenting cells (APCs) that detect antigens and initiate a wide spectrum of immune responses against them. These characteristics make them promising candidates for immunotherapy manipulations as gene carriers for cancer therapy.

Aims: To design and synthesize a targeting peptide-PEG--PEI block-copolymer that would spontaneously complex with tumor antigen encoding-DNA, and deliver it selectively into dendritic cells.

Methods: The cysteine harboring peptides DC3 (PRQPTSHYSPYF) and *scrm* (control, YPHFPSRPYQST) were synthesized by a solid phase synthesis method. A 3.5 kDa NHS-PEG-MAL and a 25 kDa linear PEI were reacted, and DC3 or *scrm* were then linked to the MAL double bond to give DC3-PEG--PEI or *scrm*-PEG--PEI. Block-copolymers and DNA were mixed for spontaneous complexation. Ideal N/P ratio for complexation was determined by gel retardation assay. Particle size and surface charge were determined by DLS and ζ-potential measurements, respectively. Specificity of DC3 to dendritc cells (DC2.4) was determined by flow-cytometry.

Results: MALDI-TOF analysis confirmed the identity of the synthesized peptides. ^{1}H-NMR spectra showed that the conjugation process of DC3-PEG--PEI and *scrm*-PEG--PEI was carried out successfully. Gel retardation assay marked N/P=8 as the ideal ratio for complexation. The complexes generated were of 100-150nm as required for IV injection. ζ-potential measurement showed reduced positive charge for PEGylated as compared to non-PEGylated particles. FACS analysis confirmed the specificity of DC3 to DC2.4 cells. Conclusions: The DC3-PEG--PEI/DNA system demonstrated all the required physicochemical characteristics for efficient gene delivery into dendritic cells. In accordance, *in vitro* transfection efficiency and safety are currently being evaluated.

Keywords: Dendritic cells, Immunotherapy, Gene delivery.

P-6

A NOVEL BIOLISTIC TECHNOLOGY FOR CONTROLLED DELIVERY OF NANO-STRUCTURED POROUS SI DRUG CARRIERS

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In this work, a novel biolistic technology, based on a pneumatic capillary gene gun, is developed for fast, accurate, and highly localized delivery of porous Si (PSi)-based nanostructured carriers and their therapeutic cargo.The particles are tailored to carry and release a model anticancer drug, Mitoxantrone, at different release profiles. The combination of the engineered particles and the biolistic technology enables the delivery of therapeutic payloads into cancerous cells at the single cell level.

Our unique biolistic setup enables using helium pressure as high as 25 psi due to an active vacuum suction that allows non-destructive delivery. The gun targets with a high precision multiple cells at once and the volume of the tissue affected by a single shot and location can be easily adjusted by varying the diameter of the gun nozzle and the accelerating pressure.

First, we delivered our PSi particles into agarose gels, which simulate soft tissue, in order to characterize and calibrate the penetration depths distribution at different He pressures and distances from the target sample. Next, drug loaded PSi particles and neat particles were successfully delivered into breast carcinoma (MDA-MB-231) cells in culture using the optimized set-up conditions. Cell viability trials following the delivery of neat-PSi particles demonstrated no damage to the cells due to the biolstic technology. A clear cytotoxic effect was detected after the delivery of drug loaded PSi particles, compared to the control plates. This study presents a new approach for highly controlled drug delivery application over time and location.

Keywords: Porous silicon, biolistic delivery, therapeutics-carrying nanoparticles.

P-7

NOVEL SOMATOSTATIN ANALOG CONJUGATES FOR PANCREATIC CANCER DETECTION

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Pancreatic cancer remains a devastating disease with a 5-year survival rate of less than 5%. This is as a result of the fact that cancer-specific symptoms occur only at an advanced stage. Recent advances in diagnostic methods and therapeutic approaches have increased the possibility of improving the existing poor prognosis. Tumor targeted delivery based on differential properties of cancer cells has emerged as a powerful methodology for the treatment and diagnosis of cancer. Somatostatin receptors are often over-expressed in many growth-control altered primary human cancers. Thus, these receptors may be employed as binding sites for the selective delivery of cytotoxic drugs and imaging molecules to the cancer cells. Somatostatin is a small cyclopeptide hormone consisting of 14 or 28 amino acids. Some synthetic analogs of somatostatin have already been used clinically for detection and treatment of neuroendocrine tumors. However, these analogs show unsatisfactory selectivity. We developed a novel fluorescent somatostatin analog (PTR 3207-86) which has unique and superior properties: Increased chemical and metabolic stability (days), high tumor selectivity (10-100 T/NT ratios), bioavailability and improved pharmacokinetics, therefore it may be utilized as an excellent targeting agent. This analog, and some other novel somatostatin labeled analogs were designed and synthesized by solid phase peptide synthesis (SPPS), based on backbone cyclization in order to achieve better selectivity and affinity towards the various receptor subtypes and to allow personalized medicine.

Keywords: Tumor targeted delivery, somatostatin analogs, solid phase peptide synthesis

P-8

TARGETED SIRNA LIPOSOMES AND NANOPARTICLES

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Osteopontin (OPN) and the fibroblast growth factor receptor 1 (FGFR1) are proteins involved in cancer development and progression, thus inhibiting their expression utilizing siRNA technology represents a promising therapeutic strategy. Successful application *in-vivo* has significant obstacles due to the short half-life of siRNA molecules, large molecular size and high negative charge density which limits their cellular uptake. A safe and effective delivery system of siRNA would be targeted nanoparticles composed of poly(lactic-co-glycolic acid) (PLGA) or lipid based liposomes, which are biocompatible and can protect the siRNA from degradation and at the same time providing effective uptake at the tumor site.

We encapsulated siRNA against FGFR1 and OPN in polymeric and lipid–based nanoparticles. Targeting to extracellular matrix, which is abundant in angiogenic sub-endothel layer of the tumor, was achieved by decorating the outer layer of the particles with a linked navigator peptide possessing high affinity to proteoglycans. Results show that the siRNA was effectively encapsulated into polymeric nanoparticles using a double emulsion system and into liposomes as a liposome-lipoplex complex. The size of the nanoparticles and liposomes was under 200nm. The targeted nanoparticles and liposomes were also linked to the fluorescent dye, BODIPY, and flow cytometry results showed effective uptake in rat primary smooth muscle cells that naturally shed proteoglycans. In ongoing in vivo studies we examine the bioactivity of the siRNA nanoparticles.

Keywords: siRNA, Nanoparticles, Liposomes

P-9

FOLATED DRUG DELIVERY SYSTEMS OF DOXORUBICIN: A COMPARATIVE STUDY

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Supramolecular drug carriers 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 widely used as targeting agent due to overexpression of folate receptor (FR) on many types of cancer cells. We compared the pharmacological activity of two drug delivery systems bearing doxorubicin (Dox) as the active drug and FA as the targeting moiety. In the first approach, two pullulan (Pull)-based conjugates were synthesised, with and without FA, namely Pull-PEG-FA-Dox and Pull-PEG-Dox. The second drug delivery system is PEGylated liposomal doxorubicin (PLD, Doxil™) and its folate version obtained by ligand post-insertion into the commercial formulation (PLD-FA).The specific binding of Pull-PEG-FA-Dox and PLD-FA to the FR was demonstrated by a competition assay using [3H]FA on FR-overexpressing human ovarian carcinoma KB cells. Internalization and cellular trafficking of the drug delivery systems were evaluated by following doxorubicin fluorescence using laser scanning confocal microscopy. Both folate-targeted systems showed a 5-fold increase in activity compared with the non-targeted carriers, in inhibiting the proliferation of KB cells in vitro. Treatment of KB carcinoma-bearing mice with both delivery systems resulted in no difference in the anticancer activity of PLD and PLD-FA, both equally efficient in reducing the tumor size, while the Pull-PEG-FA-Dox conjugate displayed enhanced activity compared to the non-targeted Pull-PEG-Dox. This study constitutes the first side by side comparison of two actively-targeted systems, polymer therapeutics versus nanoparticulate systems evaluated in the same mouse tumor model.

Keywords: pullulan prodrugs, liposomes, active targeting.

P-10

PHARMACOKINETIC AND EFFICACY STUDY OF CISPLATIN FORMULATED IN A NEW INJECTABLE POLYMER

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Injectable biodegradable polymer poly(sebacic-*co*-ricinoleic acid), P(SA-RA) is currently under development for intratumoral delivery of drugs for treating solid tumors. In the present work, formulation development, pharmacokinetic and efficacy studies of cisplatin formulated with P(SA-RA) polymer was carried out. Polymer-cisplatin formulation was prepared successfully and the pharmacokinetic data reflects the lower maximal concentrations and sustained release of cisplatin in polymer-cisplatin formulation compared to standard cisplatin administration. Further, in efficacy study a single intratumoral or near the tumor injection of polymer-cisplatin significantly reduced the tumor size compared to the standard cisplatin treatments. No toxicity as well as no macroscopic and/or microscopic changes in or near the injected area was observed, proving biocompatibility and acceptability of polymer-formulation. In conclusion, the developed formulation demonstrated controlled release and significant efficacy in delivering cisplatin and exhibits potential for further clinical development.

Keywords: Poly(sebacic-co-ricinoleic acid), cisplatin, localized delivery system

P-11

CROSSING THE BARRIER: NEW PATHWAY FOR DELIVERING MOLECULES ACROSS THE CELL'S PLASMA MEMBRANE

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The uptake of therapeutic cargo into the cell's cytoplasm requires the utilization of portals to cross the plasma membrane barrier. Naturally occurring portals involve highly regulated pathways which consist of transport systems for specific low molecular weight molecules or endocytic pathways for specific high molecular weight substances. Our recent discovery [1] of *proton-induced-uptake (PIU)*, which describe the massive de-novo creation of plasma membrane vesicles, can be exploited for an efficient delivery of various impermeable moieties into the cells, in a relatively short time. High proton concentrations at the external surface of the plasma membrane neutralize the negative charge of the phospholipids polar-heads which leads to a transversal asymmetry in surface charge density. This electromechanic disturbance in membrane deformation energy can be balanced by the membrane adapting new conformation to its spontaneous curvature with possible energetic preference to occur at pre-existing tension lines. The result, demonstrated by TEM images, is the formation of caveolae-like membrane nanostructures which rapidly recycle back to the plasma membrane, while releasing their content into the cytoplasm. The formation of these membrane invaginations is accompanied by enhanced uptake of molecules and particles (e.g. polysaccharides, polynucleotides and nanoparticles [1, 2]) into the cell interior. PIU is shown to be independent of the known pathways of receptor mediated endocytosis, macropinocytosis and membrane penetrating peptides/proteins. In vitro studies demonstrate the enhanced delivery of anti-mitotic agents to cell cultures and the insertion of naked DNA plasmids, oligo-nucleotides and siRNA into the cytoplasm.

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Keywords: Plasma membrane, Drug delivery, Endocytosis

P-12

DESIGN AND EVALUATION OF DRUG LOADED CETUXIMAB IMMUNONANOPARTICLES PREPARED USING A NOVEL LINKER MOLECULE

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Lung cancer is the leading cause of cancer-related death worldwide. With current treatments, the five-year survival rate remains low, ceiling at 16%. Active targeting of nano-sized delivery systems has emerged in recent years as an attractive approach for improved cancer treatment. The conjugation of nanoparticles (NPs) to targeting ligands promotes specific binding and internalization of the carrier cargo to cancer cells while avoiding indiscriminate drug distribution and systemic toxicity. We have designed a delivery system to target the epidermal growth factor receptor (EGFR), over-expressed in lung cancer. Oleyl cysteineamide, a novel synthesized amphiphilic linker molecule, was anchored at the interface of paclitaxel palmitate (pcpl) loaded PLGA NPs producing thiol surface activated NPs. Cetuximab, an anti-EGFR monoclonal antibody was covalently conjugated to surface activated pcpl NPs via thioether bonds, to produce cetuximab pcpl INPs with high conjugation efficiency and drug load. *In vitro* studies in A549 cells exhibited specific binding and enhanced intracellular uptake and cytotoxicity of INPs compared to non targeted NPs. *In vivo*, cetuximab pcpl INPs presented clear therapeutic efficacy in mice compared to non targeted controls. Overall results indicate the potential of this promising platform for improved lung cancer treatment.

Keywords: cancer, nanoparticles, targeting

P-13

SIMULTANEOUS CHEMOTHERAPY AND RADIOTHERAPY OF TUMORS

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The advantage of inserting a controlled release porphyrin directly into anatomically-accessible malignant tumors has enabled the Photon Activation Therapy (PAT) technique to become a potential clinical modality [1]. The two independent and simultaneous actions of the palladium-tagged porphyrin, PdTMPyP4, offer both a chemotherapeutic and radiotherapeutic benefit to cancer patients. The TMPyP4 component binds and stabilizes G-quadruplex (GQ) structures in DNA and in the telomeres, ultimately inhibiting the activation of telomerase [2]. Activated telomerase prevents the shortening of the telomeric ends of DNA and confers immortality to cancer cells [3]. A photoelectric effect can be induced in the Pd component by implanted iodine-125 brachytherapy seeds, whose energies stimulate the release of densely ionizing Auger electrons in the GQ and cause non-reparable damage to the structures. The controlled release of PdTMPyP4 is a therapeutic necessity for successful PAT because both actions of the drug require its long term presence in tumor. The c-myc oncogene is the most unstable of all oncogenes, readily copies itself, and stimulates telomerase activation. Continuous stabilization of the GQ in the promoter region of c-myc prevents its over-expression and inhibits telomerase activation. Equally necessary is the continued availability of Pd atoms for their constant release of Auger electrons. Because low dose rate brachytherapy procedures involve the interstitial placement of radioactive seeds directly into the tumor, we have developed a rod using the poly(lactide-co-glycolide) polymer PdTMPyP4/PLGA as the drug delivery system. In preliminary studies we show retardation in the growth rate of a highly chemo-resistant breast tumor model after intratumoral rod insertion.

References:

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Keywords: Photon Activation Therapy; telomerase inhibition; intratumoral injection

P-14

ANTICANCER THERAPY BASED ON NANODRUG CHEMOTHERAPY COMBINED WITH THERMAL ABLATION

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We hypothesized that combining thermal ablation and nanoliposomal chemotherapy would act synergistically to improve therapeutic efficacy of these two anticancer therapy modalities. The former anticancer treatment is based on in-situ tumor heating using radiofrequency (RF). Two different nanoliposomal formulations were fabricated and evaluated: liposomes encapsulating a combination of vincristine and topotecan as two drugs in one liposome, named LipoViTo, and liposomes encapsulating doxorubicin (Doxil®).

Nude mice were injected with either lung cancer (A549) or medulloblastoma (Daoy) cells. After 4-5 weeks, the mice developed tumors with a diameter of ~14-15 mm. Two days after treatment with RF ablation and/or chemotherapy, the mice were sacrificed. The tumors were excised and sliced to thin disks. Histopathological studies included staining for mitochondrial enzyme activity and measuring the necrosis area. Combination as modality treatment (nanoliposomes and RF) was compared to single modality treatments (nanoliposomes or RF).

For medulloblastoma, the most efficacious treatment was the combination of LipoViTo with RF, while for lung cancer it was the combination of Doxil with RF. Concentrations of drugs in tumor tissue after chemotherapy with RF were higher compared to single therapy with liposomes alone.

Survival curves for 90 days in animals bearing human medulloblastoma were determined. Both combinations, LipoViTo plus RF and Doxil plus RF, were superior in improving survival to each single treatment, chemotherapy or RF.

In conclusion, the combination of RF and nanoliposomal chemotherapy is superior in both types of cancerto RFor nanoliposomes alone. However, for optimization of the chemotherapeutic drug has to be selected according to the cancer type.

Keywords: Nanodrug, Chemotherapy, Radiofrequency

P-15

AN IMPROVED PEGYLATED LIPOSOMAL DOXORUBICIN WITH SIGNIFICANTLY LOWER PPE THAN DOXIL®

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The high demand for Doxil® during the current shortage is a good demonstration of its high utility. Doxil® has many advantages over free doxorubicin (Solomon and Gabizon (2008) Clin. Lymphoma Myeloma 8:21-34, Barenholz (2012) J. Control. Release, *in press*). The excellent drug retention, combined with the very long circulation time of Doxil® allow the liposomes to accumulate in the skin in high doses, inducing skin toxicity, a phenomenon known as "palmar-plantar erythrodysesthesia" (PPE) or "hand-foot syndrome" (Gabizon *et al.* (1994) Cancer Research 54:987-992). In clinical studies and current routine treatments at 50 mg/m^2 dosing every 4 weeks, more than 50% of patients treated with Doxil® developed PPE. The prevalence of this side effect limits the maximum tolerated dose (MTD) of Doxil® to 50 mg/m^2 (compared with 60 mg/m^2 for free doxorubicin). In this study we introduce a novel formulation of doxorubicin encapsulated in PEGylated long circulating liposomes (DOX003) designed to reduce the PPE effect. Our preliminary experiments with rats demonstrate that DOX003 shows an improved safety profile with regard to PPE and gross toxicity compared to Doxil®. Our results demonstrate that under the same treatment conditions tested and at equal doses and total amounts of doxorubicin, the treatment with DOX003 resulted in a much lower severity of PPE and toxicity compared with the rats injected with Doxil® in terms of general health (body weight, appearance) and clinical PPE symptoms. **Based on the above results (and on unimpaired efficacy), DOX003 is on its way to clinical trials.**

Keywords: liposomes, Doxil®, PPE

Abstracts of posters selected for short oral presentations

Thursday, September 6, 2012

P-16

ELUCIDATING THE MECHANISM OF INTERACTIONS BETWEEN MESENCHYMAL STEM CELL DERIVED NANO-VESICLES AND CANCER CELLS

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Introduction:

In our lab we are developing a targeted delivery system for cancer therapy, which is based on unique vesicles produced from the cell membrane of mesenchymal stem cells (MSC), which are known for their homing capability. These cell derived vesicles (CDV) benefit surface molecules of the MSC and thus may preserve their targeting mechanism. We aim to study the interactions between the CDV and cancer cells.

Materials and Methods:

CDV were prepared by sonication and extrusion and characterized for morphology, size distribution, zeta potential, and surface protein presentation using transmission electron microscopy, dynamic light scattering, flow cytometry, and mass spectrometry. The effect of an encapsulated anti-cancer drug on the viability of prostate cancer cells (PC3) was evaluated using the alamarBlue® assay.

Results:

CDV prepared from MSC had a spherical shape with both unilamellar and multilamellar structures. The average size and zeta potential of the produced CDV was 127.3±8.1 nm and -15.9±1.6 mV, respectively. Flow cytometry analysis of surface proteins presented on the produced CDV revealed that several markers were completely retained, while others were partly retained or lost during preparation. Using mass spectrometry more than 500 proteins were identified. Treating PC3 with a CDV encapsulated anti-cancer drug had significant effect on cell viability compared to the free drug in the same concentration.

Conclusions:

The CDV system benefits optimal features such as a nano-scale size and presentation of integrins involved in cell-cell and cell-extracellular matrix interactions. The feasibility of the CDV system was proven by an increased cytotoxic effect of an encapsulated anti-cancer drug on cancer cells.

Keywords: Vesicles, Mesenchymal stem cells, Cancer.

P-17

CONTROLLED PENETRATION OF HPMA-R8 COPOLYMERS INTO TUMORS USING BIODEGRADABLE POLYANIONS

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One of the greatest challenges for tumor targeted drug delivery systems (DDS) is to achieve deep and efficient penetration to all regions of the tumor while minimizing internalization into healthy tissues. A way to face this challenge is to utilize short cationic peptide sequences, named cell penetrating peptides (CPP) that can transport different payloads into mammalian cells without requiring specific receptors. The cationic amino acids, lysine and arginine are essential for cell penetration through electrostatic interactions with the negatively charged cell membrane and can be manipulated to control the activity of CPPs. Previous reports of selective CPP activation include enzymatic digestion, acidic pH and light irradiation. We report herein the development of a polymeric DDS that is based on biocompatible non-degradable N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers bearing multiple copies of the CPP octa-arginine (R8) which is electrostatically complexed with various biodegradable polyanions. The HPMA-R8 copolymer can rapidly penetrate cells only after dissociation of the coplexes. The dissociation could be either externally triggered by the addition of protamine, or attained over time using the appropriate biodegradable polyanion. HPMA-R8 containing doxorubicin copolymers (HPMA-R8-DOX) were complexed with poly-L-glutamate (PGA) and were shown to significantly prolong survival of mice bearing B16 melanoma lung metastases.

P-18

THE MECHANISM OF ULTRASOUND EFFECT ON CHORIOAMNION MEMBRANE MASS TRANSPORT

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Researchers have long been seeking and developing new noninvasive methods in every field of medicine. These methods include sonophoresis, a noninvasive method that utilizes Ultrasound (US) in order to enhance skin permeability. Previous results obtained in our lab showed that US also enhances reversibly mass transport across Chorioamnion (CA) membrane. In this study we evaluated the mechanism of US effect on CA membranes mass transport. The CA is comprised of two membranes, Chorion and Amnion (maternal and fetal side respectively). The CA membranes were exposed to potassium indigo trisulfonate (PIT) color after US exposure in two configurations: US facing Chorion or Amnion. We used two solutions, double distilled water (DDW) and degassed DDW (to minimize cavitational effects). CA membrane images of pre and post treatments were taken and analyzed using specially constructed MATLAB™ based code. The results showed that Amnion is more permeable than the Chorion. Also CA membranes exposed to US display an increase in new localized transport regions (LTRs) formation and causes pre-existing LTRs to be more permeable. We hypothesized that the Chorion is rate limiting membrane. And indeed we noticed that when the Chorion is exposed to US, the Amnion is stained almost immediately.

The use of degassed DDW resulted in a decrease in new LTRs formation, while color intensity in those LTRs remained the same. Since the CA membrane has many LTRs to begin with combined with our previous observation that US effect on CA membrane is short term, suggests that acoustic streaming is the dominant mechanism of US enhancement.

Keywords: Ultrasound, Chorioamnion membrane and image analysis.

P-19

STEM CELL DERIVED NANO-VESICLES, A NEW DELIVERY PLATFORM FOR CANCER TARGETED THERAPY

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The ultimate goal in cancer drug-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. Here, we present 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 and hypo-immunogenicity. Encompassing MSC surface proteins and armed with their unique targeting capabilities, the vesicles may be loaded with various drugs and as demonstrated *in-vitro*, can be selectively targeted against multiple cancers.

Cell derived vesicles (CDV) preparations were shown to resemble common nano-scaled drug delivery systems in size and morphology. Bio-distibution *in vivo* assay showed accumulation of CDV at the tumors, 24 hr and 1 week post IP administration. A single systemic administration of sTRAIL-loaded vesicles, made from human or rat MSC, achieved specific inhibition of human prostate tumor in nude mice demonstrating active inter-species *in-vivo* targeting.The nano-vesicles rapidly cleared from non–target organs, with neither apparent toxicities nor side effects, implying towards the safety of this system, which may be readily modified to target various MSC-susceptible cancers and other pathologies.

P-29

DEVELOPMENT OF NANO-SCALED POLYMERIC CARRIERS FOR INTEGRIN-ASSISTED DOXORUBICIN DELIVERY

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Differences in the structure and behavior of normal and tumor tissues are utilized for designing drug delivery systems (DDS) facilitating tumor-selective delivery of a drug or a prodrug . Polymer therapeutics at a nano-scaled size of approximately 10-150 nm extravasate from the tumor leaky neovasculature and accumulate selectively in tumor tissues due to the enhanced permeability and retention effect . Together with this passive targeting mechanism, active targeting strategies using ligands or antibodies directed against selected tumor determinants amplify the specificity of these therapeutic nano-scaled DDS. We designed and synthesized Doxorubicin-entrapped polylactic-*co*-glycolic acid (PLGA) nanospheres, poly(ethylene glycol)-Phe-Lys-doxorubicin (PEG-FK-DOX, cleaved by cathepsin B) and PEG-E-maleimidocaproic acid hydrazide (EMCH)-DOX (hydrolyzed in the acidic endosome) linear conjugates. Both polymers are clinically-approved, biocompatible, non-toxic and non-immunogenic polymers. These DDSs were compared in terms of hydrodynamic diameter volume distribution and their toxic effect on human and murine cancer cell lines. According to their properties, a lead compound was selected and further conjugated with an RGD peptidomimetic moiety, E-[c(RGDfK)$_2$], which actively and selectively targets endothelial and tumor cells overexpressing $\alpha_v\beta_3$ integrin. Using semi-quantitive RT-PCR technique and FACS analysis, overexpression of $\alpha_v\beta_3$ integrin was validated in HUVEC and U-87 MG cells. PEG-DOX-E-[c(RGDfK)$_2$] conjugate and free DOX exhibited similar cytotoxic effect on U-87 MG human glioblastoma cells. In addition, PEG-DOX-E-[c(RGDfK)$_2$] inhibited the proliferation of human endothelial cells and their attachment to fibrinogen-coated wells. Preliminary intravital non-invasive imaging studies using near-infrared probes revealed that a PEG-E-[c(RGDfK)$_2$]-cyanine conjugate preferentially accumulated in mCherry-labeled-DA3 murine mammary tumors inoculated orthotopically in female BALB/c mice (Polyak *et al.,Polymers for Advanced Thechnologies,*2011). Altogether, our results show a proof of principle for selective delivery of DOX to proliferating endothelial and cancer cells overexpressing $\alpha_v\beta_3$ integrin.

Keywords: αvβ3 integrin, doxorubicin, drug delivery system

P-21

TARGETED RADIOLABELED IMMUNO-NANOPARTICLES FOR TREATMENT OF HER-2/*NEU* POSITIVE CANCERS

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Taxane nanoparticles (NPs) are being investigated to improve the therapeutic treatment of various cancers. Conjugation of cell-specific ligands to NP surfaces have shown improved outcomes, however, such targeted delivery systems do not eradicate the tumors and alternative approaches are sought for improving treatment. In this study, a combined, trifunctional nanoparticulate drug delivery system (NDDS) was developed consisting of PLGA, paclitaxel palmitate, trastuzumab and a DOTA-derivative chelated radioisotope. The NP concentrations of trastuzumab, DOTA, drug and ^{111}Indium were determined as well as the particle size distribution, zeta potential, morphology and *in-vitro* drug release profile. The NDDS cytotoxicity, specificity and cell internalization were evaluated. The obtained NPs were spherical, exhibiting a mean diameter of 70nm. Trastuzumab and DOTA were successfully conjugated to the NPs surface, with a density of over 100 and 10,000 molecules per NP, respectively. FACS and laser confocal microscopy images indicated that trastuzumab affinity to the HER2 receptor did not decrease following conjugation to the NPs. Furthermore, incubation of ^{111}Indium-loaded trastuzumab-DOTA-NPs to HER2 overexpressing cells elicited increased internalization and indicated that the radioisotope chelating properties of DOTA were retained following the conjugation to NPs. Indeed, the improved drug internalization of the NDDS, probably mediated by endocytosis, resulted in an increased cytotoxic effect as observed in PC-3 cell line overexpressing the target antigen. These results show promise towards the implementation of a combined drug and radioisotope targeted delivery system that should be evaluated in animal models prior envisioning the possibility of preclinical development.

Keywords: Nanomedicine, Radioimmunonanoparticles, Trastuzumab

P-22

DETECTION OF ENDOGENOUS K-RAS MRNA IN LIVING CELLS BY MOLECULAR BEACONS

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Detection of mRNA alterations is a promising approach for identifying biomarkers as means of differentiating benign from malignant lesions. By choosing the *K-ras* oncogene as a target gene, two types of molecular beacons (MBs) based on either phosphothioated DNA (PS-DNA-MB) or peptide nucleic acid (TO-PNA-MB, where TO = thiazole orange) were synthesized and compared *in vitro* and in cancer cells. Their specificity was examined in wild-type *K-Ras* (HT29) or codon 12 point mutations (Panc-1, SW480) cell lines. Incubation of both beacons *in-vitro* with total RNA, extracted from the three cell lines, showed higher fluorescent signal detected for target (Panc-1) than in single mismatch mRNA transcripts. Cell transfection studies were conducted using the cationic polymer polyethyleneimine (PEI). Because PEI is insufficient for the formation of polyplexes with neutral backbone PNAs, TO-PNA-MB was hybridized with partly complementary DNA to form PNA-DNA hybrid with a net negative charge. This created a PNA-DNA-PEI polyplexes that, in turn, were able to transfect the tested cells with the PNA-DNA hybrid. A specific fluorescence was detected in cells expressing the fully complementary RNA transcript (Panc-1). No detectable fluorescence in cells expressing the K-Ras mRNA (single mismatch to the designed TO-PNA-MB) was observed. In contrast, PS-DNA-MB showed no fluorescence in all cell lines tested, post PEI transfection.

Based on the fast hybridization kinetics and on the single mismatch discrimination found for TO-PNA-MB, we believe that such molecular beacons are promising for in vivo real-time imaging of endogenous mRNA with single nucleotide polymorphism (SNP) resolution.

Keywords: in-vivo imaging, K-ras oncogene, molecular beacon

P-23

GLUCOSE COATED GOLD NANOPARTICLES FOR COMBINED FUNCTIONAL AND STRUCTURAL CT IMAGING

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PET-CT, which is the main molecular imaging modality in clinical use, has the great advantage of combining functional and structural imaging. PET-CT adds new clinical information and has been shown to consistently impact treatment decisions, particularly for evaluation of nodal and metastatic disease. However, PET-CT has low resolution (< 8 mm), short active timespan of radiopharmaceutical contrast-agents, complex image fusion and reconstruction, and high cost. The development of Glucose-coated gold nanoparticles (Gluco-AuNP), which serve as combined functional and structural CT imaging contrast agents, will be described. We quantitatively demonstrate, in vitro and in vivo, using Flame atomic absorption spectroscopy and CT, that Gluco-AuNP specifically target areas of increased metabolic activity and tumor cells. This development may expand the role of CT beyond its present structural imaging capabilities, endowing it with both functional- and molecular-based imaging capabilities.

Keywords: Functional imaging

P-24

HYALURONAN COATED NANOARTICLES: THE INFLUENCE OF THE MW ON CD44-HYALURONAN INTERACTION AND IMMUNE RESPONSE

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Hyaluronan (HA), a naturally occurring glycosaminoglycan, exerts different biological functions depending on its molecular weight ranging from 4000-10M Da. While high Mw HA (HMw-HA) is considered as anti-inflammatory, low Mw HA (LMw-HA) has been reported to activate an innate immuneresponse. In addition, opposing effects on cell proliferation mediated by the HA receptor CD44, have also been reported for high and low Mw HA. We have previously demonstrated that HMw-HA can be covalently attached to the surface of lipid nanoparticles (NPs), endowing the carriers with long circulation and active targeting towards HA-receptors (CD44 and CD168) highly expressed on tumors. Here we present a small library of HA-coated NPs distinguished only by the Mw of their surface anchored HA ranging from 6.4 kDa to 1500 kDa. All types of NPs exerted no effect on macrophages, T cells and ovarian cancer cells proliferation. In addition, no induction of cytokines or complement activation was observed. The affinity towards the CD44 receptor was found to be solely controlled by the Mw of the NPs surface-bound HA, from extremely low binding for LMw-HA to binding with high affinity for HMw-HA. These findings have major implications for the use of HA in nanomedicine as LMw-HA surface modified-NPs could be a viable option for the replacement of polyethylene glycol (PEG) when passive delivery is required, lacking adverse effects such as complement activation and cytokine induction, while HMw-HA-coated NPs could be used for active targeting to CD44 overexpressing tumors and aberrantly activated leukocytes in inflammation.

Keywords: Hyaluronan, Nanomedicine, complement activation

P-25

ORALLY BIOACTIVE MUCOADHESIVE INTESTINAL POLYPEPTIDE DELIVERY SYSTEMS

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One of the major challenges of therapeutic polypeptides remains the necessity of an invasive route of delivery (i.e. injection) due to poor bioavailability through non-invasive routes. Several strategies have been proposed to increase oral bioavilabilty, including permeation enhancers, enzyme inhibitors, and encapsulation technologies. However, despite significant research in the area, oral delivery of polypeptides still poses a significant challenge. Here we describe addressing this challenge with an engineering solution: developing an oral delivery system which is capable of delivering clinically relevant doses of polypeptides through the intestinal epithelium while maintaining the integrity and functionality of the intestine. This novel system consists of a mucoadhesive device specifically designed to promote unidirectional release to and absorption via the small intestine epithelium, while protecting the polypeptide from degradation in the gastrointestinal tract. Results are presented with salmon calcitonin as model drug. When calcitonin-containing wafers were incubated with PBS, the drug was released in a time-dependent manner over 5 hours, and wafers showed strong adhesion to the mucosa of porcine intestine. Release profile and adhesive strength can be controlled by modifying the composition of the wafer and backing layer. In *in vitro* studies, wafers enhanced transport of calcitonin across Caco-2 monolayers without damaging the tight junctions. *In vivo* studies with mucoadhesive wafers demonstrated a significant increase in oral bioavailability of calcitonin (C_{max} 10.3 ng/ml vs. 0.8 ng/ml with control drug solution injected into the intestine) resulting in 22% decrease in plasma calcium concentration as compared to minimal decrease with control.

Keywords: Mucoadhesive, Polypeptides, Oral delivery

P-26

EFFECTIVE SUBLINGUAL DELIVERY OF FLUMAZENIL FORMULATION FOR REVERSING THE RESIDUAL SLEEPINESS EFFECT OF HYPNOTIC DRUGS

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Residual sedative effect is a frequent side effect of hypnotics. The consequences of having residual daytime sleepiness and drowsiness are numerous like morning somnolence, decreased neurocognitive performances, and an increased risk for car or other accidents.

Flumazenil is a generic drug, extensively used for 20 years with excellent safety and efficacy profile. It binds $GABA_A$receptor with high affinity and specificity and antagonizes the sedative effects of benzodiazepines and other hypnotic drugs by competitive interaction. The current predominant clinical use of Flumazenil is in emergency rooms to reverse benzodiazepine intoxications or in operating rooms following anesthesia to counteract the sedative effect of hypnotic drugs. Flumazenil is currently available only for intravenous (IV) injection at low concentrations (0.1mg/mL solution).Following IV administration, the onset of clinically apparent effect is rapid and usually occurs within 1 to 5 minutes. The drug has a short elimination time of approximately 1 hour. Following oral administration Flumazenil is rapidly absorbed but bioavailability is low (16%) because of significant presystemic elimination.

Flumazenil was formulated using inclusion and stacking complexations to increase its solubility reaching 4mg/ml and 12mg/ml concentrations respectively. The formulations were administered sublingually using a metered-dose spray to get rapid absorption and fast onset of action. A proof of concept study with the 4 mg/ml formulation was demonstrated in a successful phase I/IIA clinical study in 20 subjects. The hypnotic effect of brotizolam and zolpidem was reversed 20 minutes following sublingual administration of flumazenil, and the effect lasted for at least 60 minutes. Having an efficient and concentrated on-demand Flumazenil sublingual spray of rapid effect might prevent the consequences of residual sedative effects of hypnotic drugs. Coeruleus Ltd. received FDA approval for a fast track 505(B)2 and plans a phase IIB clinical study under IND.

Keywords: Flumazenil, sublingual, sleepiness

P-27

PLANT CELLS AS ORAL DELIVERY VEHICLE OF HUMAN RECOMBINANT GLUCOCEREBROSIDASE FOR THE TREATMENT OF GAUCHER DISEASE

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Gaucher disease is caused by the reduced activity of a lysosomal enzyme, glucocerebrosidase (GCD). Enzyme replacement therapy (ERT) with recombinant GCD has been proven safe and effective in efficacious in treating Gaucher symptoms. To date, ERT is given once bi-weekly using IV administration. The need for oral protein formulations has been recognized by patients and researchers, though so far, no success has been reported with globular proteins. For a protein to be delivered orally, it needs to survive the GI tract and also be absorbed across the intestine to the blood stream. Plant cells have the unique attribute of a cell wall which makes them more resistant to passage through the gastrointestinal (GI) tract, and thus makes them an optimal vehicle for oral delivery of a recombinant protein without the need for extra protection. We explored the delivery of human recombinant GCD (rGCD) that was over expressed in carrot cells, via the oral route. Results show the advantage of plant cells as a vehicle to protect rGCD against degradation along the GI tract*in-vitro*. In addition, we fed rats and pigs with rGCD containing cells and monitored the absorbance of the enzyme in the blood circulation and target organs, namely the liver and the spleen. In this study we were able to show the advantage of rGCD containing plant cells for oral treatment of Gaucher disease.

Keywords: oral delivery, plant cells, Gaucher's disease

P-28

LIPOSOMES AS LUBRICANTS, A NEW APPROACH FOR OSTEOARTHRITIS – ONGOING TRIALS

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Moebius Medical is developing a lubricating, intra-articular injectable solution for diarthrosis (synovial joints).

The healthy articular cartilage is smooth and has low friction -- allowing the bones in a joint to glide smoothly over one another upon movement. The osteoarthritis cartilage is thinned, eventually completely worn out, resulting in a "bone against bone" joint, reduced motion, and pain.

We studied and compared liposomes of various lipid compositions either in the form of small unilamellar vesicles (SUV) or large multilamellar vesicles (MLV) using a cartilage-on-cartilage model that has been developed in order to assess the lubrication capabilities and wear and tear reduction

DMPC/DPPC-MLVs was found to be the best bio-lubricant and the best anti-wear protector in these models (Sivan *et al.* 2009, Verberne *et al.* 2010). The mechanism of action of these liposomes involves hydrophilic lubrication related to high level of PC head group hydration at the LD phase and its unique softness at temperatures slightly above the SO to LD phase transition temperature (Sivan *et al.* 2009). Intra-articular injection of radiolabeled of DMPC/DPPC-MLV demonstrated prolonged durability (more than 28 days) in the joint (Dolev *et al.* in preparation). Local toxicology studies proved that intra-articular injection of a high dose of DMPC/DPPC-MLV in rabbits and rats is safe. A series of studies according to ISO-10993 confirmed its high biocompatibility.

A "First in man" study of this DMPC/DPPC-MLV is now being conducted at Hadassah Medical Center / Orthopedic Department.

Keywords: osteoarthritis, bio-lubricant, DMPC/DPPC-MLV

P-29

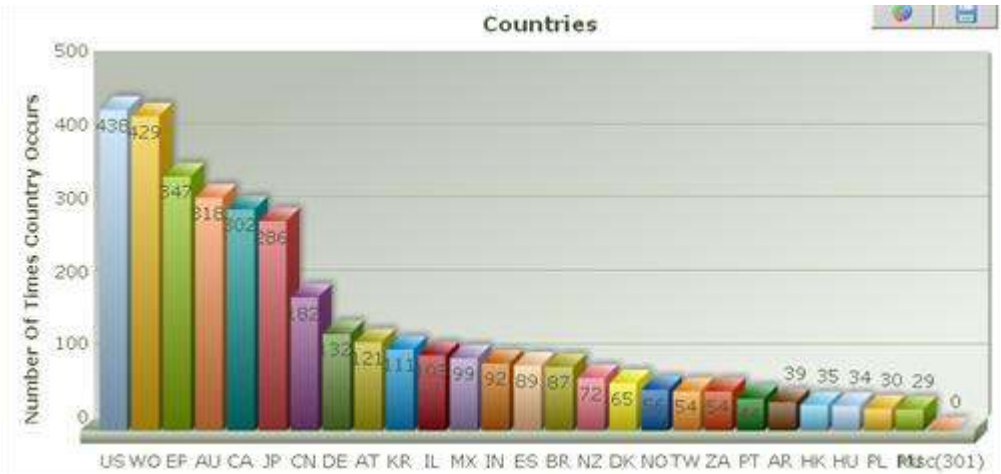
PATENT PROTECTION FOR INNOVATIONS IN ANTI-ANGIOGENIC THERAPY

Joseph Wyse, Keren Hagai
Patents, Dr. Eyal Bressler Ltd., Israel

Anti-angiogenic therapeutics are one of today's most rapidly advancing areas of medical research. New nano-drug approaches promise new applications. 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? 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 in the field, and strategies are suggested for researchers to maximise patent value and financial benefit of future advances in the field. The patenting position of Israeli research groups is particularly emphasised in our analysis of patent data. 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 discussed.

Anti-Angiogenic Therapy, Patenting Trends:



Keywords: patent nano anti-angiogenic

P-30

BREAK-THROUGH IN THE PROCESSING AND CONTROLLLING OF BIODEGRADEABLE MICRO-CAPSULES AS DRUG CARRIRERS

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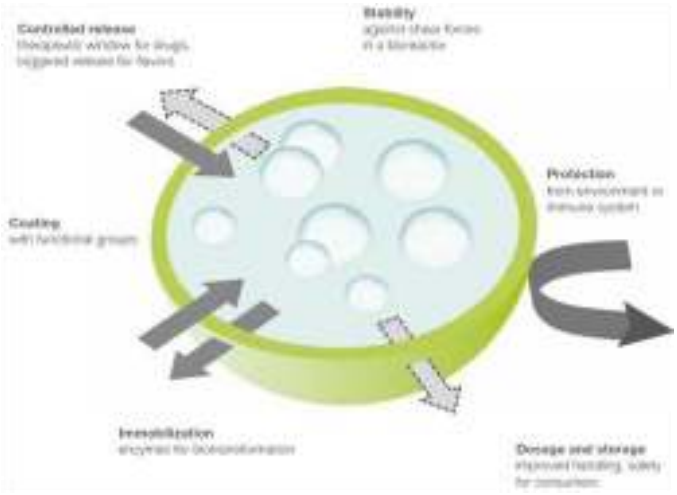
Application, Dr. Golik, Israel

Micro-Encapsulation is a powerful tool in the area of controlled drug delivery. This technique can be applied to slow and controlled release of drugs, protection of active compounds from immune systems, and to solubility enhancement and phase transfer. This straightforward and flexible method was specially designed for the immobilization of drugs, enzymes, cells, microbes and vitamins in biodegradable polymeric capsules. These capsules can be produced from a large variety of polymers such as Alginate, Gelatin, Chitosan and in different sizes and shapes. The major principles of micro-Encapsulation will be presented along with case studies which emphasize the importance of it. A new technique for processing and controlling Micro-capsules will be introduced. This technique is of great importance for the pharmaceutical and food research and development departments and for the chemical and biological research in academia.



Key words: Micro-Capsules, Drug delivery, Controlled release.

Abstracts of Posters

P-31

PHARMACOKINETIC-PHARMACODYNAMIC MODELING ANALYSIS OF THE OPTIMAL LOCAL DELIVERY RATE OF TNF-α-NEUTRALIZING ANTIBODIES IN RHEUMATOID ARTHRITIS

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Introduction: Anti-TNF-α antibodies that are used to manage rheumatoid arthritis (RA) can interact with TNF-α in the synovial fluid and in other locations in the body. The relative contribution of these local vs. systemic anti-TNF-α effects has been analyzed recently for adalimumab using a model with three sites of antibody-TNF-α interaction[1]. Based on this analysis, intraarticular sustained release formulations can improve the balance of local vs. systemic effects, as compared to the systemic administration of the drug.

Objectives: To estimate the optimal local delivery rate of TNF-α-neutralizing antibodies in rheumatoid arthritis using intraarticular sustained release formulations.

Methods: The available data from the scientific literature on the variability in the RA disease state and the pharmacokinetics of TNF-α-neutralizing antibodies were analyzed using a target-mediated drug disposition (TMDD) model with three sites of antibody-TNF-α interaction. Dose-response of intraarticular adalimumab in the individual patients and its dependence on the individual variability factors have been analyzed.

Results: Intraarticular adalimumab administration using sustained release formulations can improve the balance of local vs. systemic anti-TNF-α effects. Based on the average data, the optimal rate of intraarticular adalimumab delivery (the therapeutic window) was in the range of 4.4-14.1 pmol/hr/L. The disease state (the baseline TNF-α secretion rates in the individual compartments) was the highest source of inter-patient variability in the anti-TNF-α effects of adalimumab. As a result, the optimal local delivery rate of adalimumab in the individual patients ranged from 0.3-4.0 to 52-66 pmol/hr/L.

Conclusions: Inter-patient variability of the disease state substantially affects the therapeutic window of anti-TNF-α antibodies. Therefore, sustained release formulation that continuously releases anti-TNF-α antibody at a certain pre-set rate will lead to suboptimal responses in high proportion of the individual patients. Adjustment of drug dosage based on the individual variability factors is required to optimize the balance of local vs. systemic anti-TNF-α effects.

References: 1. Stepensky D. Local vs. systemic anti-TNF-alpha effects of adalimumab in rheumatoid arthritis: pharmacokinetic modeling analysis of interaction between a soluble target and a drug. Clin Pharmacokinetics, in press, 2012.

P-32

DEVELOPMENT OF ANALYTICAL APPROACHES FOR QUANTITATIVE CHARACTERIZATION OF NANO-DRUG DELIVERY SYSTEMS INTENDED FOR INTRACELLULAR TARGETING

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Background: Many biopharmaceuticals act on intracellular targets and should reach the site of action in specific organelle in order to exert pharmacological effect. Due to the limited permeability and stability of biopharmaceuticals, efficient delivery requires encapsulation of these agents into specialized drug delivery systems targeted to the intracellular site of action. We devise new approaches of targeted delivery of antigenic peptide to the endoplasmic reticulum of the antigen-presenting cells for anti-cancer vaccination.

Objectives: 1) development of endoplasmic reticulum (ER)-targeted delivery system, based on nanoparticles (NPs) loaded with antigenic peptide and surface-decorated with peptidic targeting residues. 2) analytical characterization of the developed system and assessment of the targeting residues' conjugation efficiency.

Methods: Antigenic peptide-loaded PLGA-based NPs were prepared using a double emulsion technique and were decorated using a novel 3-stage conjugation approach. We applied/developed a panel of analytical tools (HPLC, spectroscopy and imaging-based) to assess the amount and stability of the surface-conjugated targeting residues, drug encapsulation efficiency and kinetics of drug release.

Results: We have generated spherical NPs with desired morphology, encapsulation efficiency, and cargo release kinetics. Following individual conjugation steps, we observed characteristic changes of the NPs' FTIR spectra indicating successful conjugation process. Decoration with targeting residues affected the uptake and intracellular trafficking of NPs and effectiveness of these processes was dependent on the type of the cells used in the experiments.

Conclusions: The applied analytical tools allow quantitative analysis of the developed NPs and their optimization (to enhance the drug encapsulation capacity and efficiency of decoration with the targeting moieties). The optimized NPs and the developed analytical tools will be used to assess the feasibility of intracellularly-targeted delivery, to identify the primary parameters that limit the targeting efficiency, and to develop delivery systems that can efficiently reach the target organelles and release their cargo in a controlled fashion.

P-33

TOXICITY ASSESSMENT OF COMPOUNDS RELEASED FROM DISPOSABLE MEDICAL DEVICES USING A NEW *IN VITRO* ASSAY

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Introduction: Disposable medical devices (DMDs) release potentially toxic extractable materials during their use. *In vitro* safety assessment of DMDs is usually performed using L-929 mouse keratinocytes. However, cells that are present in the patient's blood vessels are predominantly exposed to the extractables that reach the systemic circulation and may be more suitable for the safety assessment of DMDs.

Objectives:

1) To develop a new *in vitro* method for safety assessment of DMDs based on the mouse cEND brain endothelial cells.

2) To apply this method for toxicity assessment of extractables from intravenous infusion sets of different design from a variety of manufacturers.

Methods: We analyzed infusion sets from different manufacturers that varied in design and storage time. cEND cells were incubated with extracts of individual parts of the infusion sets (tube, cup, latex), and relative toxicities were analyzed using MTT test, DCFH-DA-based analysis of reactive oxygen species formation, apoptosis and cell cycle analyses.

Results: We identified a pattern of yellowing of the infusion sets upon storage and revealed that it originated from the latex part. Extracts of the individual parts of the infusion sets were toxic to the cEND cells leading to enhanced formation of the reactive oxygen species, inhibition of the cells' metabolic activity, alterations of the cell morphology, induction of apoptosis and cell death. The MTT test and the light microscopy were the most informative parameters for *in vitro* toxicity assessment of the studied extracts.

Conclusions: Infusion sets release extractables that can be toxic to the endothelial cells of the patients that receive infusion. cEND mouse brain endothelial cells appear to be appropriate for *in vitro* analysis of infusion sets toxicity and we suggest to use them to analyze the safety and stability of infusion sets and other medical devices that release extractables into the systemic circulation.

References:

Dakwar GR, Kaplun V, Kojukarov L, Gorenbein P, Schumacher I, Kontorovich D, Förster C, Beit-Yannai E, Stepensky D. Toxicity assessment of extracts from infusion sets in cEND brain endothelial cells, Int J Pharmaceut 2012, in press.

P-34

A SIMPLE NOVEL APPROACH FOR THE CALCULATION OF "PACKING PARAMETER" VALUES OF AMPHIPHILES

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Amphiphiles can associate into a variety of structures in aqueous solution. The shapes of these aggregates are determined by geometric constrains of their hydrophilic "heads" and hydrophobic "tails" cross areas. Packing parameter (PP) is defined as the ratio between the hydrophobic and the hydrophilic cross area of the molecule, and it determines the expected shapes of the aggregates that form[1]: PP<0.67 is characteristic for micelle forming amphiphiles, 0.67<PP<1.0 is characteristic for liposomes, and amphiphiles with PP > 1 self-aggregate into inverted phases. The packing parameter is not generally calculable *a priori*. The experimental measurement of the packing parameter is not simple since it requires expertise in X-ray diffraction techniques[2] and surface tension measurements of amphiphiles at the air/water interface[3], both methodologies require special instrumentation. We created a database of different amphiphile molecules with their packing parameters, which we found in the scientific literature. For each of the molecules the following data was estimated from their chemical structure: number of carbon chains, the length of the carbon chains, number of double bond kinks, number of H-bonds, number of ions at pH 7, number of atoms that are bound to a charged atom, and number of stable ions. The PP was calculated using a non-linear regression of multiple variables, which takes into consideration these parameters. Our simple algorithm can predict a PP that is close to the PP determined experimentally in various publications. Even when the algorithm deviates from the real PP, it does not confuse between micelles, liposomes, and inverted phases.

P-35

THERAPEUTIC ULTRASOUND WAVES FOR THE DELIVERY OF GENES INTO CELLS: MECHANISTIC STUDIES

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Background

The mechanisms by which therapeutic ultrasound (TUS) mediates gene delivery into cells remains poorly understood. Understanding the mechanism by which TUS delivers genes into cells, its bioeffects on cell membrane, the kinetics of DNA trafficking to the nucleus and its time of expression are crucial in order to improve transfection efficiency and allow better control of this modality.

Methods

Experiments were performed to evaluate the effects of TUS (if exist) on: (1) the cytoplasmatic membrane, (2) DNA intracellular trafficking and (3) nuclear transport. In these experiments the effects of different factors on TUS mediated transfection were studied.

The effect of TUS on the viability of the cells was measured using the AlamarBlue® assay. The effect of TUS on cell morphology, DNA transport and kinetics in the cells and on the nucleus were also studied.

Results & Conclusions

Our results imply that TUS mediated transfectionis a complex process which involves several different mechanisms. Endocytic pathway as well aspore formation,play an important role in TUS mediated transfection. DNA intracellular traffickingmay include pathways that rely on both microtubules and actin fibers.

TUS did not affect the viability of the cells. In-situ Time-laps imaging revealed that during TUS application the nucleus undergoes reversible morphological changes.

Keywords: Gene Delivery, Mechanism, Ultrasound.

P-36

DEVELOPING POLYMERIC DELIVERY SYSTEM FOR THE ENTRAPMENT OF INSULIN-PRODUCING STEM AND LIVER CELLS.

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Introduction

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

Methods

Hum-Hep cells and human mesenchymal stem cells (hMSC) were transduced using recombinant adenovirus containing PDX-1 gene. Transduced cells were encapsulated in alginate-PLL mixed with ECM isolated from pancreatic tissue. The effect of encapsulation parameters on cell viability and insulin secretion was followed using AlamarBlue and C-peptide secretion, respectively.

Results

Encapsulated cells were viable for more than 120 days. Moreover, the morphology of the encapsulated cells showed that they grow and spread in their surrounding especially around micro pieces of ECM. C-peptide measured 5 days after initial exposure to the viral transduction, revealed that the encapsulated hMSC had significantly higher secretion levels than non-encapsulated hMSC.

Conclusion

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

Keywords: Cell encapsulation, Mesenchymal stem cells, Extracellular matrix.

P-37

NUMERICAL SIMULATION OF THERMOSENSITIVE LIPOSOMES DELIVERY TO TUMORS USING HIGH INTENSITY FOCUSED ULTRASOUND

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The efficacy of cancer chemotherapy can be greatly enhanced by targeting thermosensitive drug-containing liposomes using high intensity focused Ultrasound (HIFU) (Dromi *et al.,* 2007). We have developed a theoretical model of mass transport describing the spatial and transient drug distributions in epithelial mesenchymal transitions (EMT) and epithelial tumors. In this model, the effects of HIFU on increasing the tumor microvasculature permeability (Watson *et al.,* 2012) and liposomes' rupture (Dromi *et al.,* 2007) are considered. In addition, we also took into account the influence of tumor cell apoptosis and necrosis on drug transport into the tumor (Zhang, Mi, Yang, & Xu, 2009). The model predictions agree well with published experimental results (Watson *et al.,* 2012). Thus, the model has been used to simulate nano-sized liposomes drug distribution in the tumor post HIFU application. Simulation results show that HIFU enhances liposomes accumulation in the tumor. Accumulation is greatest when the time between HIFU treatment and liposomes injection was less than one hour. Moreover, HIFU shortened greatest liposomes accumulation time compared to untreated (control) tumors from 26 h to 15 h. This model can be used to predict the treatment outcome and optimize the clinical protocol.

Keywords: HIFU, liposomes, mathematical model

P-38

LAMININ DERIVED PEPTIDE – POLYMER CONJUGATE FOR SELECTIVE TARGETING PACLITAXEL TO CD44 EXPRESSING CANCER CELLS

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Background: Conventional chemotherapy is limited because of its non-specificity, poor pharmacokinetics and multi-drug resistance. We have hypothesized that CD44 targeted polymer conjugates would increase therapeutic effect of Paclitaxel (PTX) owing to the passive tumor accumulation and rapid internalization into CD44 overexpressing cells and could further have the potential to overcome drug efflux pumps due to different (receptor-mediated) mechanism of cell entry, when compared to free drug.

Methods: A CD44-targeted delivery system based on the water-soluble N-(2 hydroxypropyl)methacrylamide (HPMA) copolymer was designed. The polymer possesses the laminin based peptide A5G27 as a ligand for targeting the CD44 cell-surface receptor, associated with a wide variety of tumors and the metastatic spread of cancer. FITC-labeled HPMA copolymer precursor having active ester groups for peptide attachment was first synthesized by radical precipitation polymerization. A5G27 was conjugated to the copolymer precursor via p-nitrophenyl (ONP) aminolysis. ^{1}HNMR was used to characterize the copolymer. The weight average molecular weight (Mw) and polydispersity (I) of the copolymer were determined by SEC. ONP and FITC content was determined using spectrophotometry. The binding and internalization of A5G27 containing copolymers to CD44-expressing cells was determined by means of flow cytometry.

Results and Discussion: The binding of the targeted HPMA copolymer bearing A5G27 peptide to CD44-overexpressing cells *SK-OV-3* and *B16-F10*, was significantly higher relative to the control, non-targeted copolymer. The ability of the CD44 targeted polymer PTX conjugate system to overcome P-gp resistance in P-gp overexpressing cell line is currently being investigated.

Keywords: HPMA, CD44, A5G27

P-39

INJECTABLE GELS BASED ON PORCINE CARDIAC EXTRACELLULAR MATRIX FOR MINIMALY INVASIVE DELIVERY OF CELLS TO INFRACTED HEART.

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Introduction

Cardiovascular disease continues to be the leading cause of death, suggesting that new therapies are needed to treat the progression of 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 heat based gel that can serve as an injectable scaffold for human induced pluripotent stem cells (hIPSC) for myocardium rejuvenation.

Materials and Methods

Gels were prepared by solubilyzing decellularized porcine cardiac extra cellular matrix (pcECM) and studying its combination with polymers such as chitosan in different concentration and cross linker genipin. The gels were than characterized by scanning electron microscopy (SEM), and mass spectrometer. The mechanical properties were measured with plate reometer. hIPSC were cultivated within the gels and viability, proliferation and differentiation towards the cardiac linage were evaluated using Alamar Blue assay and Immunofluorescent staining.

Results

Gels contained mostly collagen (more than 70%) were assemble in the form of thin fibers mesh (down to 5nm width). Mechanical strength of the gels elevated by increasing chitosn and genipin concentrations and all the gels showed support of cultivation of embryonic bodies derived from hIPSC.

Conclusions

Injectable material based on pcECM offers the potential for minimally invasive cells delivery to the heart. Furthermore the ability to cultivate hIPSC and their derivative within such construct may bring unique value to the cardiac engineering field of personalized medicine.

Keywords: cardiac, injectable gel, extracellular matrix.

P-40

FOCUSED ULTRASOUND TRIGGERED POLYMER DEGRADATION, AN APPROACH FOR NONINVASIVE RESORPTION OF THE INFERIOR VENA CAVA FILTER

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Deep Venous Thrombosis (DVT) is a pathological condition when micro-thrombi form blood clots within the deep veins of the thighs. When a blood clot detaches from the vessel wall and travels in the blood stream (Embolism), it risks blocking the blood flow in small blood vessels. The life-threatening complication of a DVT is Pulmonary Embolism (PE) that occurs when a pulmonary arterial blood vessel is blocked. Inferior Vena Cava (IVC) filter is a temporary medical metal device inserted by catheterization to the IVC, physically preventing the passage of large blood clots to the heart and lungs. We propose a new approach to determinate the filter functionality in a non-invasive manner, in which metal wires are connected by a degradable polymer joint, degraded on demand after exposure to Focused Ultrasound (FUS) radiation.

PLGA polymers are biodegradable and FDA approved for therapeutic applications, their strength and ability to connect metal wire suit our demands. In our studies of FUS radiation effect on PLGA properties we found FUS increases PLGA degradation rate due to both cavitation and thermal effect. After FUS radiation we have observed significant change in PLGA sample shape and structure; macro scale by naked eye and micro scale by scanning electron microscopy (SEM). In SEM we observed structure changes in the micron range and porosity in the nanometer range. However, thermal properties of the polymer are not affected by FUS radiation as no change in glass transition (Tg) point has been observed. Our goal is to develop a suitable method of ultrasound-triggered polymer degradation for IVC filter opening.

P-41

THERAPEUTIC ULTRASOUND FOR GENE DELIVERY TO MESENCHYMAL STEM CELLS -TARGETED ANTI-ANGIOGENIC TUMOR THERAPY

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Background

Genetically engineered stem cells, such as mesenchymal stem cells (MSC), is an exciting therapeutic concept that offers the promise of therapy for an array of disorders and malignancies. MSC have been demonstrated to migrate to tumors and ischemic tissue, thus can be used as a carrier of therapeutic DNAs. Ultrasound (US) is a non-viral approach used to deliver genes into cells and tissue. Non-viral vectors, which are considered safer, easier to prepare and lack of immunogenic response are emerging as substitutes to the viral ones.

Methods & Results

MSCs were transfected with pGFP using therapeutic-US (TUS) and ultrasound contrast agents (USCA) achieving over 30% transfection. The TUS parameters, such as time, duty cycle (DC) and intensity were chosen after optimization of the TUS system. Biological activity of TUS-transfected secreted PEX: pDNA encoding for PEX (an anti-angiogenic factor) was added to the MSCs and were exposed to 1 MHz TUS at 20% DC, 2 W/cm^2 for 20 min with USCA. Conditioned media was taken from TUS-transfected MSCs and was added to human prostate cancer cells (PC3), primary endothelial cells (HUVEC) and MSCs. Viability was measured using AlamarBlue®.

Conditioned media taken from TUS-transfected MSCs with pDNA-PEX affected the migration of HUVEC and reduced the viability of PC3 and HUVEC cells by 40% and 20% respectively, While not affecting the viability of the MSCs. Currently we are in process of *in-vivo* studies investigating the efficacy of such an approach on tumor growth.

Conclusions

Our study demonstrates that TUS can efficiently transfect MSC while maintaining their viability. The TUS-transfected MSCs secrets the PEX to their media and reduces the viability of prostate cancer cells while sparing non-cancer cells. These results suggest that transfected MSC may be used as a carrier of therapeutic DNAs in order to migrate to tumors and ischemic tissue.

Keywords: Gene Delivery, Mesenchymal Stem Cells (MSC), Ultrasound.

P-42

CELL DERIVED VESICLES AS A NEW VEHICLE FOR DELIVERY OF siRNA

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Introduction

Drug delivery systems are vastly explored and developed using synthetic polymers as vehicles. We are developing a novel system in which vesicles derived from cellular membranes serve as vehicles. We have produced cell derived vesicles (CDV) from mesenchymal stem cells (MSC), known for their ability to migrate to tumors. Membrane proteins on the surface of MSC are key factors in the migration process. Therefore, the membrane mosaic may retain part of this ability and serve as a carrier for drug delivery to tumors. We aim to use this system in order to carry siRNA to cancer cells.

Materials and Methods

CDV were prepared by sonication and extrusion and characterized for size distribution and zeta potential, using dynamic light scattering. For analysis of siRNA content, PAGE and fluorescence measurements were conducted.

Results

The average size and zeta potential of the produced CDV was 156.3nm and -15.1 mV, respectively. siRNA did not degrade during the preparation process, as revealed using PAGE assay.

Future work

Morphology of vesicles and loading of siRNA will be characterized using cryo-TEM and fluorescence measurements respectively. Silencing will be tested on prostate cancer 3 (PC3) cells, using real time RT PCR analysis, and protein gel analysis.

Keywords: Vesicles, Mesenchymal stem cells, siRNA.

P-43

ULTRASOUND EFFECT ON DNA PERMEABILITY THROUGH AMNIOTIC MEMBRANE

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In recent years, many biomedical studies have been devoted to finding non-invasive medical procedures. One of the methods being used is ultrasound, which is known to enhance biological membranes' mass transport phenomena ([1]Mitragotri, S. and J. Kost, Advanced drug delivery reviews, 2004).

Amniocentesis and chorionic villus sampling (CVS) are invasive prenatal tests offered to pregnant women and possess a risk of miscarriage (up to 2% for CVS). This arouses the need for noninvasive procedures that retain the same specificity and sensitivity as the invasive procedure ([2]Tabor, A. *et al.,* Ultrasound in Obstetrics & Gynecology, 2009).

The aim of this research is to evaluate ultrasound effects on the amniotic membrane, in order to enhance its permeability to fetal DNA, thus enable sampling of amniotic fluid (specifically fetal DNA) in a noninvasive manner in order to detect genetic abnormalities. Results obtained previously in our lab demonstrated that chemical penetration enhancers (CPEs) combined with ultrasound enhanced the permeability of fluorescent model drug (FITC Dextran, 70 kDa) across amniotic membrane by 87 fold.

In this study,*in-vitro*experimentswere performed on post-delivery human amniotic membranes (authorized by the Helsinki committee of Hillel Yaffe Medical Center, Israel) in order to evaluate the enhancing effect of ultrasound on plasmid DNA (pEGFP, Escherichia coli Green Fluorescent Protein, 3000kDa) permeability across amniotic membrane.

Preliminary experiments were performed with fluorescent model molecule (2000kDa) of the same size of expected fetal DNA fragments. The results demonstrated that ultrasound alone increased the mass transport of the fluorescent model molecule across amniotic membranes compared to control experiments. Work protocols for preliminary experiments with pEGFP and for evaluating DNA enhancement with polymerase chain reaction (PCR) are currently being developed in our lab.

Keywords: amniotic membrane, ultrasound and DNA.

P-44

ULTRASOUND INDUCED INCREASED SKIN PERMEABILITY OF siRNA FOR PSORIASIS TREATMENT

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Psoriasis is a chronic skin disease, caused by rapid and incomplete differentiation of skin basal cells that affects 2-5% of the general population compromising patients' quality of life. The treatments offered today provide only a short time relief. Recently, it was discovered that specific small interfering RNA (siRNA) injection into basal cells results in increased apoptosis of these cells and cessation of the rapid differentiation [G. Lerman et al, British Association of dermatologists 164, 2011]. siRNA is a short sequence of RNA, specifically designed to attach to specific pare of the messenger RNA for the silencing of desired protein. One of the main challenges is finding a method for efficient delivering of siRNA into the basal cells. Since skin is permeable to molecules smaller than 500Da, delivering siRNA (13 kDa) to skin cells needs to utilize methods which enhance transdermal delivery. Sonophoresis is a non-invasive method in which application of ultrasound increases the permeability of skin. [S. Mitagotri, J. Kost Drug Delivery Reviews 56 (2004) 589-601]. The aim of this research is to develop a non-invasive method for delivering siRNA molecules into the basal skin cells using ultrasound as a mean to enhance transdermal delivery. The results demonstrated that ultrasound (I=7.14 W/cm2, f=20 kHz, duty cycle 50% and 45 min duration) indeed increased the permeability of the fluorescent model molecule (FITC-Dextran, 70 kDa) across porcine skin compared to passive diffusion. In addition, fluorescent microscope images of 5 microns thick porcine skin exposed to FITC -Dextran 70 kDa and treated with ultrasound(I=7.14 W/cm^2, f=20 kHz, duty cycle 50% and 5 min duration) showed that the model molecule penetrated all epidermal layers, even into the basal cell and into the nucleus.

Keywords: Psoriasis, siRNA, Sonophoresis

P-45

DEVELOPING A CELL-DERIVED NANO-VESICLES BASED SYSTEM FOR THE TREATMENT OF HEART INFLAMMATION

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Ischemic heart diseases affect millions of people in the western world. Among them is myocardial infarction (MI), usually followed by inflammation in the heart tissue leading to the formation of scar tissue and often results in congestive heart failure. Our lab has developed a novel targeted drug delivery system, which is based on unique vesicles produced from the plasma membrane of mesenchymal stem cells (MSCs), known for their homing abilities. These cell-derived vesicles (CDVs) maintain surface proteins of the MSCs and thus may preserve their targeting potential. We aim to study this novel system's ability to target inflammation in heart tissues, thus possibly minimizing infarct size and improve healing via interactions with various cell types resident in the inflammatory environment, including cardiomyocytes, macrophages and myofibroblasts. Experiments were designed to study the interactions of these vesicles with HL-1 cells, derived from mouse atrial cardiomyocyte tumor lineage, which exhibit contraction and retain differentiated cardiac morphological properties even during passaging. As for the macrophages, we have been working with THP-1 cell line and study the targeting of vesicles to these cells. For the myofibroblasts we have received cells isolated from a MI model in sheep. The CDVs prepared from hMSCs had a spherical shape, with an average size and zeta potential of 127.3±8.1nm and -15.9±1.6mV, respectively. Flow cytometry analysis of surface proteins showed the presence of some. The novel system of the CDVs carries optimal features such as a nano-scale size and the presence of surface proteins such as integrins involved in cell-cell interactions.

Keywords: Vesicles, Mesenchymal stem cells, myocardial infarction.

P-46

DEVELOPING A MECHANICAL AND ELECTRICAL STIMULATION APPARATUS IN A PERFUSION-BIOREACTOR FOR RESEEDED DECELLULARIZED PORCINE ECM/H1

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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 show to support and direct cell growth, differentiation and the overall tissue functionality. A bi-functional apparatus providing mechanical and electrical stimuli was incorporated into a perfusion bioreactor that was reported by us before to support the survival of mesenchymal stem cells, endothelial cells and cardiomyocytes reseeded onto decellularized pcECM. The high mechanical pressure required on the interior side of the heart wall is provided by a balloon placed under the tissue that is inflated and deflated using a syringe pump connected with a linear actuator mimicking the circulatory beating. The electrical action-potential, of the left ventricle, is simulated using an electrical output device attached to carbon electrodes placed into the perfusion chamber and creating an alternating electrical field. To achieve precise balance between the two rhythms, a computer-controlled input/output device collects data and controls both stimuli while monitoring other parameters such as the pH, oxygen, temperature etc.

Future applications of this system, that are supported by preliminary results, 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.

Keywords: Cardiac tissue engineering, mechanical stimuli, electrical stimuli

P-47

ENGINEERING THICK PORCINE EXTRACELLULAR MATRIX FOR CARDIAC REGENERATION

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Cardiac Tissue Engineering has been receiving much attention as an important approach for cardiac regeneration, mostly following myocardial infarction. Whereas most studies demonstrated the use of thin cardiac scaffolds (<1 mm) to patch the infarcted area, we have recently reported the production of thick acellular scaffold (> 10 mm) for replacing the entire left ventricular wall which is made from decellularized porcine extracellular matrix (pcECM). The obtained cardiac patch, having clinically relevant dimensions and resembling the native tissue, can be used to replace most of the infarcted tissue area and reduce the fatality associated with end-stage heart failure. Mesenchymal stem cells (MSC) harboring regenerative potential (and a common model for cardiomyocytes) were shown to attach, survive and grow on decellularized pcECM treated with nitrocellulose compared to proteinaceous and other chemical treatments. The reseeded MSCs retained linage integrity as evident by their constitutive expression of typical MSC markers: CD90, CD105, CD44 and CD73. Dynamic culturing under perfusion was shown to support cells survival for up to 21 days which also lead to cell infiltration much deeper into the tissue bulk (>0.2 mm) compared with static culturing (<0.1 mm). Preliminary results further support future prospects of combining reseeded cells on treated pcECM with growth factors and in co-culture with cardiac fibroblasts to produce a more functional reseeded pcECM that will be soon evaluated *in-vivo*.

Keywords: Cardiac Tissue Engineering, Thick cardiac patch, Recellularization

P-48

DEVELOPING CELL-DERIVED VESICLES AS A GENE DELIVERY VEHICLE FOR CANCER THERAPY

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Introduction

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 its microenvironment. The cell derived vesicles (CDV) will benefit the surface molecules of the MSC and thus may preserve their targeting mechanism. Into this "Trojan horse" CDV system we will incorporate therapeutic encoding DNA, which will be expressed at the tumor site.

Materials and Methods

CDV were prepared, PEGylated (to produce stealth CDV) and characterized for morphology, size distribution, zeta potential and surface protein. CDV- PC3 (prostate cancer cells) interaction was evaluated by Flow cytometry and confocal microscopy. DNA was complexed with different cationic agents (to overcome electrostatic repulsion between the DNA and the cell membrane) and incorporated into the CDV. DNA Incorporation efficacy was evaluated as well as the physical characteristics of the DNA encapsulating CDV. CDV-pGFP expression was evaluated post incubation with PC3 cells, using Flow cytometry.

Results

CDV preparation resulted in round vesicles with an average size of 180nm and a zeta potential of about -16 mV (-12 mV for PEGylaed CDV). Flow cytometry analysis demonstrated preservation of most of the MSC surface markers. CDV showed specific and time-dependant accumulation inside the cytoplasm and nucleus following incubation with PC3 cells. pGFP was successfully incorporated to the CDV system, and preliminary experiments demonstrate expression of GFP in PC3 cells after a 6 hour incubation.

Keywords: Cell Derived Vesicles, Mesenchymal stem cells, Cancer gene delivery.

P-49

DEVELOPING VASCULARIZATION IN CARDIAC DECELLULARIZED PORCINE ECM GRAFT USING A BIOREACTOR

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Vascularization remains a critical requirement for the long term survival of engineered tissue constructs, especially thick ones. Due to the lack of oxygen and nutrient supply, cells cannot survive with diffusion distance of more than 100μm away from the nearest blood vessel [1]. Such thick constructs for cardiac tissue engineering has been reported by our group [2] and others based on decellularized porcine cardiac extracellular matrix (pcECM) that has been shown to resemble the native tissue both structurally and chemically. The network of inherent vasculature which was largely retained within our pcECM, can be used as a platform for re-endothelialization [3] and neo-vascularization utilizing cell lineages with regenerative potential. Sequential co-cultures of endothelial cells (HUVEC) and mesenchymal stem cells (MSC) were shown to support the growth of both lineages on the surface and in the vasculature of reseeded pcECM. Endothelial cells alone, seeded into the vasculature of chemically treated ECM (and grown under dynamic conditions), not only attached and survived but also rearranged into typical confluent monolayer morphology. The medium, seeding densities and cell ratios used in the co-culture experiments, were optimized in a 2-dimensional co-culture system, revealing a "prey and predator" interactions between the co-cultured cells that correspond with the Lotka-Volterra model. Preliminary results showed that future efforts combining co-culture, treated scaffolds and growth factors may not only result in re-endothelialization but also lead to the sprouting of new blood vessels and neo-vascularization.

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Keywords: vascularization, stem cell, extracellular matrix

P-50

TARGETING GLIOBLASTOMA WITH AN ANTICANCER MICRORNA-PG-AMINE POLYPLEX

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Glioblastoma multiforme (GBM) are aggressive primary neoplasms of the brain that exhibit notable refractivity to standard treatment regimens. Recent large-scale molecular profiling has revealed deregulated molecular networks as potential targets for therapeutic development. MicroRNAs (miRNAs) are noncoding RNA molecules which act as post-transcriptional regulators of specific messenger RNA transcripts. miRNAs play major roles in normal developmental processes, and their deregulation significantly contributes to various aspects of carcinogenesis.

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 its stability, intracellular trafficking and silencing efficacy. Polyglycerol (PG)-Amine, a water-soluble polyglycerol-based hyperbranched polymer accumulates in the tumor microenvironment due to the enhanced permeability and retention (EPR) effect, and therefore, represents an ideal delivery vehicle for antitumor biological agents (Ofek P. *et al.*, The FASEB Journal, 2010).

Using our novel nanocarrier, we have studied the expression targets and functional effects of several anticancer miRNAs in several human glioblastoma cell lines. The miRNAs levels inversely correlated to their target gene levels measured in the same cell lines. Transient transfection of the anticancer miRNA-PGNH_2polyplex into glioblastoma cell lines strongly inhibited cell proliferation, cell cycle progression, and cell migration.

Together, our findings show that PG-Amine is able to deliver anticancer miRNAs to glioblastoma cells and to suppress brain tumor growth by downregulating their validated targets. These results suggest that our polyplex could serve as a potential therapeutic.

Keywords: microRNA, PG-Amine, glioblastoma

P-51

INCREASING ORAL BIOAVAILABILITY OF ACTIVE PEPTIDES: AFFECTING INTESTINAL PERMEABILITY OF CYCLIC HYDROPHILIC PEPTIDES BY MULTIPLE N-METHYLATION

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Oral administration of peptide therapeutics has high potential for treating various diseases. However, their oral bioavailability is problematic due to low membrane permeability and high metabolic degradation. Cyclosporine-A is a highly permeable cyclic peptide, with seven N-methyl groups. It was hypothesized that an increased degree of N-Methylation (i.e. replacement of a hydrogen in the amino acid's amide bond by a methyl group) would improve the permeability of hydrophilic peptides via its impact on their lipophilicity. To examine this hypothesis, a library of 54 multiple N-methylated, cyclic poly-alanine hexapeptides was synthesized, differing in the number and positions of N-methylations in the peptide sequence. The library included all possible varieties of N-methyl combinations. The peptides underwent permeability screening using Caco-2 cells, $PAMPA_{lecithin}$ and the Ussing chamber models. Permeability results through the Caco-2 monolayer revealed highly variable permeability among the different subgroups. E.g., Papp values that varied from $0.86*10^{-6}$ to $30.8*10^{-6}$ cm/sec for two tetra-N-methylated peptides. Interestingly, both the mono- and penta-N-methylated groups consistently showed low permeability. Our study indicated that the position of N-methylations and the consequent conformation, rather than their number, is a key factor in dictating the peptide's permeability characteristics. Thus, the permeability of the peptides across lipid barriers could not be predicted by their physicochemical properties, as was previously believed. The high permeability properties obtained in several N-methylated analogues demonstrates that this approach provides a solution for improving oral bioavailability of hydrophilic peptides, thus enabling the conformational prediction and design of bioactive peptides for oral administration.

Keywords: Oral bioavailability, Peptide, N-methylation

P-52

PRO-NANOLIPOSPHERE (PNL) FORMULATIONS FOR IMPROVED BIOAVAILABILITY AND ADVANCED BIODISTRIBUTION: ACTIVITY OF BLANK NANO PARTICLES

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Purpose: While incorporation of lipophilic drugs into PNL was usually considered to improve their oral bioavailability by enhancing solubilization, we have shown that additional mechanisms are involved in this process. These include: reduced intra-enterocyte metabolism and/or P-glycoprotein (P-gp) efflux inhibition. In this study we aim to elucidate whether utilization of drug free particulate systems (blank-PNL) may also elevate the oral bioavailability of these drugs.

Methods: Blank-PNL and amiodarone (AM)-PNL were developed. PK of AM was assessed *in-vivo*. The effect of blank-PNL on P-gp was assessed *in-vitro* (Caco-2 cells) using talinolol (Pgp marker). The effect on intra-enterocyte metabolism was evaluated in microsomes using testosterone as positive control.

Results: Oral administration of AM-PNL and AM+blank-PNL resulted in similar oral bioavailability. Co-administration of talinolol+blank-PNL *in-vitro* yielded a significantly higher permeability coefficient vs. talinolol alone (1.73×10^{-6} and 7.66×10^{-7}, respectively). In addition, a significant difference (1.3 folds) in intact testosterone concentrations was found following incubation of testosterone + blank-PNL *versus* testosterone in microsomes.

Conclusions: These results demonstrate that co-administration of blank-PNL with a drug has the same effect on its bioavailability as the incorporation into the PNL. In both cases the drug bioavailability is increased significantly to similar extent. This unique finding suggests that the drug delivery system optimization for maximal drug load capacity is no longer required. Hence, advantage of such a novel mode of administration is that blank-PNL can be separately administered in a capsule in close proximity to the intake of the active compound, as a generic solution for improving oral bioavailability of these drugs.

Keywords: bioavailability, Pro-NanoLipospheres, intra-enterocyte metabolism

P-53

NOVEL COCHLEATES FOR NANOMEDICINE: FORMATION AND STRUCTURE

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Lipids are fascinating building blocks that can be shaped into various structures, phases and mesophases, and used for numerous medical purposes. Examples include vesicles entrapping and stabilizing anti cancer drugs (e.g., Doxil), drug-loaded cubosomes and hexosomes for topical applications, and lipid-DNA complexes (lipoplexes) used for gene therapy. The lipids serve as mediating agents that stabilize, mask and transport the encapsulated compound into the host cell.

In lipoplexes, lamellar and hexagonal mesophases are formed through electrostatic interactions between positively charged lipids and DNA, stabilized by entropic effects through the release of counterions (Danino *et al.*, 2009). Cochleates represent a similar class of lipidmicrostructures, consisting of negatively charged phospholipid bilayers rolled up into cigar-like spiral rolls through the interaction with multivalent counterions (Ca^{2+}or Mg^{2+}) as bridging agents.

Using similar concepts we recently developed a new class of nanocochleatedelivery vehicles, based upon encapsulating active antimicrobial agents of the OAK (oligo-acyl-lysyl) family (Radzishevsky *et al.,* 2008) together with antibiotics into a multilayered lipid matrix, designed to simultaneously deliver both drugs, safely and effectively (Livne *et al.,* 2010). This co-encapsulation strategy was shown to synergistically reduce the minimal inhibition concentration (MIC) and improve the antibiotic effect, thus may serve as a new medical tool for overcoming resistance to antibiotic.

In the present study we explore the conditions required to engineer efficient and stable nanocochleates consisting of different species of therapeutic agents with a variety of lipid mixtures mimicking the cytoplasmic membrane of bacteria, and the biophysical characteristics of these assemblies.

Keywords: Drug-Delivery, Lipids, Nanocochleates

P-54

CONTROLLED-RELEASE INJECTABLE SUSPENSION FOR AMOXICILLIN THERAPY IN VETERINARY MEDICINE

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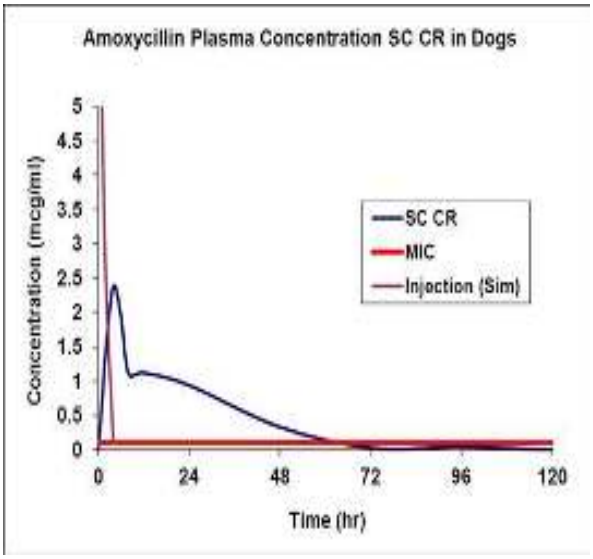
Oral administration of medications, which is generally considered a preferred route in human medicine, is, for obvious reasons, commonly unfeasible in veterinary medicine, especially when large domestic animals (such as cattle) are concerned. For similar reasons, any medication requiring multiple dosing might also prove difficult, if not impractical.

Beta-lactam antimicrobials, commonly used in both veterinary and human medicine, generally present short biological half-lives, whereas their activity is enhanced as pathogen exposure is prolonged. These properties require multiple –dose regimens of standard dosage-forms, thereby hindering pet-owner adherence, frequently resulting in therapeutic failure.

This study presents a novel controlled-release injectable drug delivery system for beta-lactams in which a single-dose administration provides the effective antimicrobial course, optimizing pharmacokinetic-pharmacodynamic profiles, minimizing adverse effects and emergence of antimicrobial resistance and facilitating adherence.

Our prototype controlled-release injectable suspension (CRIS), based on thermosensible poloxamers, was designed to enable continuous input of these drugs to their absorption site over several days. Several CRIS formulations of the beta-lactam amoxicillin were evaluated in in-vitro dissolutions studies. The optimal formulation was selected for further in-vivo canine studies, for determination of pharmacokinetic-pharmacodynamic profiling.

Prolonged release of amoxicillin from the CRIS allowed the maintenance of effective drug concentrations (MIC x 4) for treatment against many clinically-relevant pathogens for more than 3 days. The developed CRIS is most feasible for veterinary medicine. This is true for large domestic animals (such as cattle), as well as for pets and other animals. It offers significant advantages over standard immediate-release therapy in achieving pharmacokinetic-pharmacodynamic goals. The prototypical formulations represent a novel platform which can be modified to meet various clinical requirements.



Keywords: Poloxamers, Beta-lactam , veterinary medicine

P-55

CONTROLLED DELIVERY SYSTEM TO ELICIT ANTI CANCEROUS PROPERTIES OF THE HIV PROTEASE INHIBITOR NELFINAVIR

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Nelfinavir is an HIV protease inhibitor that has been used to treat AIDS patients since 1993. It has the strongest anti-cancerous properties due to its ability to inhibit Akt phosphorylation which is a crucial step in cancer cell's survival, growth and resistance to anti-cancer therapy [1]. For the treatment to be effective, Nelfinavir needs to be in a constant specific concentration close to the tumor, and it needs to be administered to the body in a way that it prevents serious side effects.

The aim of this research is to develop a controlled delivery system of Nelfinavir based on Poly (lactic-co-glycolic) acid (PLGA), and test it as a treatment for cancer. Since PLGA is a biodegradable polymer that dissolved in glycofurol and solidifies upon contact with aqueous media, it may be injected directly to the tumor to form Nelfinavir controlled delivery implant that degrades at a known rate maintaining an effective concentration of the drug in the tumor.

In-vitro experiments were performed in order to examine the effect of controlled release system of Nelfinavir on cell's viability, using C6 brain cancer cell line in a tumor-like environment. The cell's viability decreased gradually and reached 30% of control within 120 hours of exposure to Nelfinavir (2.1 mg Nelfinavir per 100 μL implant). The influence of Nelfinavir and PLGA implant's concentration on C6 cell's viability is currently done in our lab.

Keywords: Controlled delivery system, Cancer, Nelfinavir.

P-56

NOVEL ORAL LOCAL SIROLIMUS SUSTAINED RELEASE DELIVERY SYSTEMS FOR THE TREATMENT OF ORAL PREMALIGNANCY

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Oral squamous cell carcinoma (OSCC) accounts for the majority of oral and pharyngeal cancer cases and characterized by a poor prognosis. One of the significant alternations exhibited by OSCC is the over activity of the mTOR (mammalian target of rapamycin) signaling pathway, a central regulator of cell growth and proliferation in response to environmental stimuli. Unfortunately, modern medicine has no response for the prevention of easily detectable oral premalignant lesions that develop into cancer. Clinicians define this situation as "watchful waiting". Sirolimus is an immunosuppressive agent acting through the mTOR pathway blockade. Studies in cell types and animal models indicate that sirolimus can be used successfully as a chemopreventive and treatment agent in cancer and particularly in OSCC. Topical application and topical SR systems, along with prolonged targeting of the drug have the advantage of reducing the required dose and significantly reducing the systemic absorption thus leading to a decrease in incidence of adverse effects as well as enhanced patient compliance. Nondegradable hydrophobic polymer based varnish sustained release (SR) drug delivery systems were developed to enable the prolonged release of sirolimus at the oral cavity. The drug delivery systems were evaluated in vitro for sirolimus release (figure 1).
The proposed study may lead to a breakthrough in chemoprevention of OSSC and thus contribute greatly to basic and clinical research done in the field as well as to patients' prognosis and quality of life.

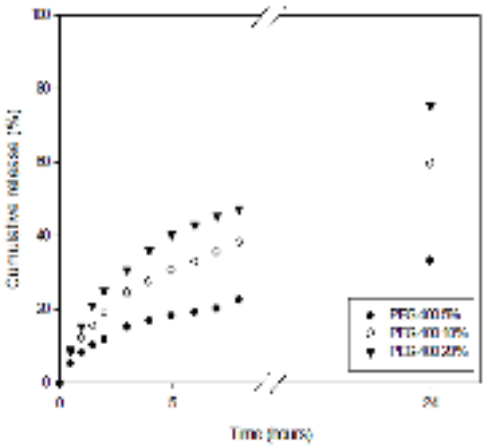

Figure 1: Cumulative release profiles of sirolimus from varnish SR drug delivery systems containing different concentrations of PEG 400.

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Keywords: Oral cancer, Sustained release drug delivery, Chemoprevention

P-57

APICAL PROTEINS EXPRESSION IN INFLAMED MUCOSA, AS POSSIBLE TARGETS FOR DIRECTING DRUG THERAPY IN IBD

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Purpose

The enterocytes of the intestinal epithelium may lose their polarization due to a variety of pathological conditions, one of which is inflammatory bowel disease (IBD). *In vitro* studies have shown that a leaky mucosal barrier in IBD patients is associated with proinflammatory cytokines, such as IFNγ and TNFα. This instigated us to explore whether the remodeling of mucosal barrier can be exploited for drug targeting under inflammatory conditions.

Methods

The levels of the basolateral proteins were monitored by immunofluorescence of human biopsies or of CaCo2 monolayers. The effect of induced inflammation on proteins levels at the surface of colonic epithelial cells was assessed by FACS in Caco2 cells that were incubated with a series of proinflammatory cytokines. Fluorescently-tagged immunoliposomes were prepared by conjugating to antiTfR antibody. Their size distribution, phosphate content, zeta potential, antibody density and specific interaction and cellular uptake in Caco2 cells, in the presence of proinflammatory cytokines, were characterized. *In vivo* analysis of the liposomes' capability to specifically accumulate in the colonic epithelium was assessed in DNBS colitis induced rats.

Results

(a) Inflammation increased TfR expression in the colonic mucosa in both apical and basolateral aspects of colonocytes.

(b) Ecadherin and β1integrin were relocated to the apical membrane during inflammation in the colonic mucosa of IBD patients.

(c) AntiTfR immunoliposomes adhered better *in vivo* to the inflamed mucosa compared to nonspecific immunoliposomes.

Conclusion

Inflammation could cause a relocation of basolateral membrane proteins in colonocytes towards their apical membrane. This trait could be exploited for directing targeted drug carriers such as immunoliposomes to the inflamed gut mucosa via the luminal route.

Keywords: IBD; Immunoliposomes; Transferrin receptor

P-58

THE RELATIVE ROLES OF CHARGE AND A RECOGNITION PEPTIDE IN LUMINAL TARGETING OF COLORECTAL CANCER BY FLUORESCENT POLYACRYLAMIDE

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The goal of the present study was to (***a***) prepare a fluorescent vehicle, at the near IR range, made of cationized polyacrylamide (CPAA) aimed at targeting the overexpressed sialic acid in colonic malignant cells and tissues; () increase the polymeric vehicle targeting capabilities by the addition of a recognition peptide (EPPT1) that targets the transmembrane glycoprotein underglycosylated MUC-1 (uMUC-1).

CPAA with increasing charge densities was prepared by radical polymerization of acrylamide and different mol % ratios of *N*-acryloyl, *N*'- (*tert*-butyl-carbonyl) diaminoethane. The NIR fluorophore, IR-783, was attached to the CPAA to give CPAA-783. After selecting the optimal IR-783 mol % ratio that avoids quenching, the specific attachment of the polymer was tested in SW-620, SW-480, HT-29, and LS-147T cancer cells. The optimal polymeric product was tested *in situ* in gut sac preparations of the dimethylhydrazine (DMH) induced rat model.

CPAA-783 preferentially bound to the CRC cells, depending on CRC staging. The best binding was observed when the fraction of the cationic monomer was 100 mol %, labeled with 0.75 mol % of IR-783. An increase in the recognition of the dually labeled polymeric product, CPAA-783-EPPT1, towards HT-29 and LS-174T cells (over expressing uMUC-1) was observed for the lowest EPPT1 molar ratio only (0.63 mol %). In the orthotopic mouse model the effect of the additional EPPT1 to the preferential binding of the CPAA-783 polymer to the malignant tissue in the colon was negligible.

Keywords: Colon cancer, cationized polyacrylamide, near IR imaging

P-59

N-PALMITOYL CERAMIDE DI (CARBAMOYL-SPERMINE) (PCDCS): A NOVEL CATIONIC SPHINGOLIPID FOR NUCLEIC ACIDS TRANSFECTION.

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Here we describe the use of N-Palmitoyl Ceramide di (Carbamoyl-spermine) (PCDCS) as a novel cationic component of *in vitro* and *in vivo* transfection reagent, a novel cationic lipid that was designed as a result of Prof. Barenholz collaboration with Bio-Lab Ltd and produced by Bio-Lab Ltd. This molecule has two primary amines that are positively charged in neutral pH and four secondary amines that could be only partially positively charged at neutral pH while being fully charged at acidic pH. These chemical properties of lipid enable it on the one hand to bind efficiently to negatively charged nucleic acid molecules and to bind the anionic plasma membrane of mammalian cells via electrostatic interactions and, on the other hand to act as an effective "proton sponge" at the endosomes where the complexes end up after internalization into cells, thereby facilitating effective "endosomal escape". We used the PCDCS for preparing the cationic liposome with the helper lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) that was shown to improve the lipoplex transfection efficiency and therefore it appears to be a favorable helper lipid for DNA transfection *in vitro*. We used this PCDCS/DOPE formulation both for plasmid DNA and siRNA *in vitro* transfection in different cell types. Our results show that PCDCS/DOPE (1:3 molar ratio) liposome has a high potential as a transfection reagent both for siRNA and a plasmid DNA *in vitro*. Moreover, our preliminary results show that PCDCS/DOPE formulation effectively knocked down Luciferase protein expressed in C26 murine carcinoma cell line *in vivo*.

Keywords: Lipid, Lipoplex, siRNA

P-60

DEVELOPMENT AND IN-VITRO ASSESSMENT OF ERODIBLE RAPAMYCIN LOCAL ORAL SUSTAINED-RELEASE DELIVERY SYSTEMS FOR PREVENTION AND TREATMENT OF ORAL CANCER

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Oral Squamous Cell Carcinoma (OSCC) is one of the most common cancers in the world, and its poor prognosis is partly attributed to the lack of efficient means for prevention and early treatment. Such means could significantly improve the survival rate. One of the most important changes that occur during the pathogenesis is the over-activation of the mTOR (Mammalian Target of apamycin) kinase. Rapamycin acts as an inhibitor of mTOR and is approved as a drug for prevention of transplant rejection. Studies have found rapamycin to be a promising agent for prevention and treatment of OSCC.[1] Rapamycin is commonly administered systemically, and is known to cause several serious adverse effects. Local sustained-release applications provide an efficient way to prolong the time of residence of the active agent in the site of action, while significantly reducing adverse effects.[2] The objectives of the current study were to develop erodible mucoadhesive sustained-release delivery systems of rapamycin for local application and to perform *in-vitro* assessment of selected systems.

A delivery system in the form of an erodible mucoadhesive tablet was selected. Several delivery systems exhibited an optimal release rate of 6-8 hours, and combining the polymers Carbopol and carboxymethyl cellulose at different proportions in the matrix was found to be the most effective tool for controlling the release rate of rapamycin.

In addition, systems containing Carbopol and sodium carboxymethyl cellulose demonstrated a high mucoadhesive performance. In summary, several erodible sustained-release delivery systems of rapamycin were developed during the study, and the key factors which allow us to control the behavior of the systems were identified, in an attempt to create a basis for a rational design of novel rapamycin applications for the prevention and treatment of OSCC.

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P-61

NOVEL ORALLY BIOAVAILABLE INSULIN SENSITIZER DRUG (*MEDICA*): THE INSULINMIMETIC ACTIVITY OF SYNTHETIC LONG CHAIN FATTY ACID ANALOGS

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Background: Slow-release insulin analogs improve glycemic control in diabetic patients but promote weight gain, inflammation markers, increase blood pressure and cancer risk. Thus, lowering the amount of exogenous insulin administered to diabetic patients has significant clinical advantage. Synthetic fatty-acid analogs (Methyl-capped Di-Carboxylic Acids (MEDICA)), previously developed by our group, exploit the "allosteric" activity of fatty acids in targeting Hepatocyte nuclear factor 4α (HNF4α), AMP-activated Protein Kinase (AMPK), Forkhead Transcription Factor O1 (FOXO1) and signal transducer and activator of transcription 3 (STAT3), while avoiding the substrate role of natural long-chain fatty acids in generating fat and energy. MEDICA analogs act as insulin sensitizers in hyperinsulinemic state (type2 diabetes).

Objectives: Current study aims to evaluate the insulinomimetic activity of MEDICA analogs in type1 diabetes context.

Results: MEDICA was orally administered (15 mg/kg BID) to Streptozotocin (STZ) diabetic rats (Fasting Blood Glucose (FBG) > 500 mg/dl) for 14 days. FBG was normalized in 40% of the MEDICA-treated rats. Non-responsive were further treated with a combination of MEDICA and limiting insulin. MEDICA treatment combined with low dose NPH insulin (3 μg/kg BID) (totally ineffective in normalizing FBG in STZ diabetic rats) has normalized FBG in all non-responsive rats. MEDICA-treated animals showed profound improvement in metabolic parameters- water consumption, urination, glucose tolerance and insulin tolerance.

Conclusions: The insulinomimetic activity of MEDICA may offer an improved insulin treatment modes for both, type1 and type2 diabetes.

Keywords: MEDICA

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DEVELOPMENT OF β-CASEIN BASED NANOCARRIERS FOR ORAL DRUG DELIVERY

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β-Casein (~24 kDa) is a milk protein that can be considered as an amphiphilic diblock copolymer. As such, β-casein above its CMC self-assembles into nanometric micelles, constructed of a hydrophobic core and a hydrophilic corona [1, 2]. In the presence of guest molecules the β-casein co-assembled into nano-assemblies whose structure is dependent on the physico-chemical properties of the guest molecules. Previously we described the development and characterization of the β-casein micellar nanocapsules as a platform for solubilization and oral delivery of water-insoluble molecules, particularly drugs and nutraceuticals [1-3]. As a natural food product β-casein is defined as GRAS (generally recognized as safe), it is easily degradable in the body and does not provoke an immune system response. Encapsulation by self-assembly within β-casein nanostructures considerably improves drug solubility, stability and shelf-life [3].

We have screened a number of guest molecules including drugs, proteins and other biomolecules - hydrophobic, hydrophilic and amphiphilic. This enables better understanding of the nanocarriers assembly and structure. We found that amphiphilic drugs, particularly celecoxib, can be encapsulated with a high mole loading ratio. In fact, the capacity of celecoxib in β-casein micelles is high enough to meet the recommended daily dose (for more details on the utility of the β-casein-Celecoxib micelles see our collaborators abstract and poster: *Hadas Perlstein et al*. ICRS 2012). Moreover, the nano-carriers can be freeze-dried, stored for long periods, and retain their structural characteristics upon rehydration. The ability to obtain a stable dried powder by freeze-drying is a great advantage for clinical use [1].

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Keywords: Beta-casein, micelles, drug delivery

P-63

TARGETING NCAM-EXPRESSING TUMORS WITH POLYMER THERAPEUTICS

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Neural cell adhesion molecule (NCAM) expression was shown to be associated with more aggressive biological behavior, increased metastatic capacity and expression of stem-cell markers in several tumors1. NCAM was found to be a marker for cancer stem cells in Wilms' tumor2. Conjugation of drugs to a polymer allows specific delivery to the tumor and provides an ideal platform for a combination treatment, since both drugs are given simultaneously and share the same pharmacokinetic profile3. We aim to target NCAM-expressing tumors using a polymeric drug delivery system, consisting of an NCAM targeting moiety, paclitaxel and doxorubicin conjugated to polyglutamic acid (PGA) backbone. Conjugate with a targeting moiety was fluorescently labeled and shown to bind NCAM-expressing ES2 ovarian carcinoma cells, as opposed to non-targeted PGA. NCAM-targeted PGA-paclitaxel conjugate exhibited higher cytotoxicity to ES2 cells compared to non-targeted PGA-paclitaxel conjugate and inhibited capillary-like tube formation of endothelial cells. Conjugate of PGA bearing a combination of doxorubicin and paclitaxel was synthesized and characterized. The combination of free drugs was shown to have synergistic effect on ES2 cells and MDA-MB-231 human mammary carcinoma cells. PGA-paclitaxel-doxorubicin conjugate displayed similar cytotoxic activity to that of the free drugs on these cells and inhibited capillary-like tube formation and migration of endothelial cells.

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Keywords: Cancer stem cells, NCAM, Polymer therapeutics, combination therapy

P-64

RECOGNITION OF SECRETED CANCER-ASSOCIATED BIOMARKERS IN THE FLUIDS OF THE GI TRACT: PAVING THE WAY FOR *IN VIVO* DETECTION OF PANCREATIC CARCINOMA

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Malignancies, for which survival has not been improved substantially over the past 30 years include lung and pancreatic cancer (PC). The latter is the fourth leading cause of cancer death in the United States[1]. The major reason for this stagnancy in PC is the lack of early diagnosis modalities (in contrast to breast and colorectal cancers). Although serum biomarkers for the early detection of PC have been reported[2], none could serve as reliable prognostic or predictive diagnostic tools.

In the present study, which deals with the analysis of the secreted biomarkers,CA 19-9 and CEA, a novel method is suggested for their detection in the small bowel fluids by a combination of an endoscopic means (e.g. modified version of the Pillcam®video capsule)[3] and a composite diagnostic system.

Here we describe a diagnostic system with the capability of (*a*) capturing and detecting selected biomarkers in a specific manner and (*b*) **detecting the derived specific optical signal at the NIR range. The design includes "Detecting" and "Sensing" compartments.**The latter consists of a Super Mask Super Amine based Sensing platform, made of microarray-type functionalized glass, modified with a series of the PEG spacers. Grafting the PEGylated surfaces with either aCA19.9 or aCEA antibodies made them capable of specifically capturing the correspondent biomarker. The novel polymer-based Detecting platform comprises of an erodible superporous hydrogel matrix, crosslinked with modified albumin as a biodegradable "brick", with the capability of entrapping and then releasing, at a constant rate, NIR-labeled immuno nanoparticles (FluoNP). These FluoNP are capable of detecting the captured biomarkers by virtue of the relevant antibodies conjugated to their surface.

In a series of detection studies, we found that CEA was detected by the novel system in both simulated intestinal fluids containing physiological concentration of trypsin, and aspirated human intestinal fluid. The signal-to-noise ratio was high enough to accomplish real-time *in vivo* detection of CEA (above the limit of a video capsule bench-simulator), indicating on the system's ability to serve as an *in vivo*, real-time diagnostic kit for PC.

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Keywords: Pancreatic cancer, biomarkers, diagnostics, hydrogel

P-65

THE ANTI-INFLAMMATORY POTENTIAL OF NOVEL CYCLOPENTENYLPHOSPHONATE IN THE LOCAL TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Rheumatoid arthritis, asthma and inflammatory bowel disease (IBD) are chronic inflammatory processes, out of which the latter is the most difficult to treat in a local specific manner. In chronic inflammation monocytes are recruited in large numbers into the site of inflammation, at which place they extravasate and differentiate rapidly into macrophages that, in turn, play an important role in the conversion of inflammation from acute phase into chronic phase, by virtue of their involvement in the continuous secretion of cytokines[1]. It has been indicated that vinylphosphonates could reduce inflammation by lowering TNF-α levels. We therefore synthesized a homologous series of novel fused-cyclopentenone phosphonates and screened them for their ability to reduce secreted TNF-α from LPS-activated macrophages. In these studies a lead compound, P-5, was identified as possible anti-inflammatory agent. Diethyl 3-nonyl-5-oxo-3,5,6,6a-tetrahydro-1H-cyclopenta[c]furan-4-ylphosphonates (P-5), was synthesized, using an intramolecular Pauson-Khand Reaction (PKR) with Mo(CO) as the catalysts and DMSO as a promoter, from diethyl 3-(allyloxy)dodec-1-ynylphosphonate. The structure and stereochemistry were determined both by 1D and 2D NMR and elemental analysis. The IC_{50} of P-5 (calculated from the reduction of TNF-α level) towards the activated macrophages was found to be 5μM. In addition, the lead compound reduced the levels of the cytokines IL-6, IL-12p70, IL-1α and INF-γ, as well as the levels of the chemokines MCP-1, MIP-1α and RANTES. These findings supported the work hypothesis that P-5 has an anti-inflammatory activity. Using 5-ASA as a positive control drug (dose of 67mg/ml), P-5, at a dose of 10mg/kg reduced inflammation in the DNBS induced-colitis model after intra-colonic administration.

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Keywords: IBD, Phosphonate, Inflammation

P-66

BETA-CASEIN NANOCARRIERS OF CELECOXIB FOR IMPROVED ORAL BIOAVAILABILITY

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β-Casein (bCN, ~24 kDa) is a milk protein that due to its amino acid composition and sequence behaves like an amphiphilic di-block copolymer and self-assembles into nanometric micelles, constructed of a hydrophobic core and a hydrophilic corona [1]. We took advantage of this property and developed β-casein micelles as a platform for solubilization and oral administration of drugs of poor water-solubility as demonstrated for celecoxib (Cx) [2,3].

As a natural food product bCN is definedas GRAS (generally recognized as safe), it is easily degradable in the body and does not provoke an immune system response. We demonstrated high Cx-loading capacity in the bCN nanostructures by self-assembly. The Cx-loaded bCN nano-carriers (bCN/Cx) can be freeze-dried and upon rehydration they retain their structural characteristics, with a slight reduction in the amount of Cx encapsulated [2,3]. A preliminary pig study performed recently showed that following oral administration of bCN/Cx as dry powder, the bioavailability of Cx was increased by 2.4-fold compared to commercial Cx oral formulation (Celebra®). It is assumed that the dry micelles reconstituted in the pig intestinal fluids, increased Cx solubilization which, in turn, increased the amount of Cx available for absorption at the epithelium surface.

Our future objectives are to perform studies in additional pigs to demonstrate and investigate the mechanism(s) of the observed bioavailability enhancement.

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Keywords: Beta-casein, micelles, drug delivery

P-67

CONTROLLED RAPAMYCIN RELEASE FROM SURFACE CRYSTALLIZED ELUTING STENT

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Coronary stenting has revolutionized current perspective of coronary artery disease management. Intense work on stent development has successfully led to the introduction of drug-eluting stents (DES) in 2002. This work presents a carrier-free DES, based on crystalline drug coating (Figure 1). The work presents rapamycin as model drug which is a macrolide and used to prevent organs rejection and also found to have significant anti-proliferation properties.

Rapamycin crystals onto stent gradually released the drug over a period of a several weeks in buffer media. Rapamycin crystal coating displayed stability and biocompatibility. Additionally, the controllability of crystallization process enables the generation of a variety of morphologies, physical states and coating thickness. In vivo experiments did not raise any obvious safety concerns, no evidence for the presence of necrosis or any inflammatory reaction. This process was further implemented using different drugs and supersaturated systems.

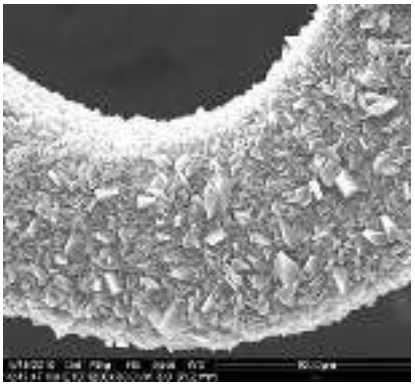

Figure 1. Rapamycin crystals onto metal stent.

Keywords: Drug eluting stents, Rapamycin, Crystallization

P-68

ULTRASONIC VELOCIMETRY OF LIPOSOMAL FORMULATIONS

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Precise measurements of ultrasonic velocity in lipid vesicles for thermodynamic and compositional characterization of liposomes are reviewed. In contrast to ultrasonic spectroscopy, in which measurements should be done in a wide range of frequencies, ultrasonic velocimetry of biological materials does not need frequency-dependent measurements, and the acoustic information on molecular structures and interactions can be obtained using measurements at a fixed frequency. The main purpose of ultrasonic velocity measurements is usually the evaluation of elastic properties of the lipids and liposomes and contributions of various kinds of intra- and intermolecular interactions. Such measurements, which started in the mid-1970s, have been widely used recently to obtain unique insights into hydration phenomena, and elastic properties of lipid assemblies, as well as for their partial specific volume, adiabatic compressibility and molecular free volume at liquid-ordered [*Sivan et al.,* 2010, *Khazanov et al.,* 2008]. Ultrasonic velocity is a useful empirical parameter employed to monitor processes in which hydration or elasticity of molecules is perturbed. The development of portable instrumentation for ultrasonic velocimetry proved the applicability of this technology for characterization of liposomal formulations, for compositional analysis of lipids and for highly sensitive liposomal immunoassay. The acoustical method for determination of critical micellar concentration can be used for complicated multi-component biological fluids and does not need dyes or fluorophores, which may affect the determination [*Priev et al.,* 2002].

P-69

DRUG ELUTING ELECTROSPUN POLYURETHANE VASCULAR GRAFTS

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Damage or stenosis of a vessel caused either by injury or by some pathological processes, e.g. atherosclerosis and thrombosis, must often be treated by replacing the vessel with an autologous graft, mostly vein.

The aim of this study was to develop a perivascular grafts releasing rapamycin as antiproliferative drug in a controlled manner. Rapamycin (RM) was incorporate into polyurethane (PU) based fibrous vascular graft either during it fabrication using electrospinning of RM-PU blends or by loading of the drug by absorption the drug into drug free electrospun PU grafts. RM-PU fibrous scaffolds were electrospun from RM-PU solutions containing 0, 1, 5, 10, 20% RM (w/w) via three distinct blending methods and the in vitro drug release was investigated. Grafts morphology and structure integrity was traced by microscopic methods. Rapamycin was constantly released for over 77 days.

Keywords: Vascular grafts, Rapamycin, Electrospinning.

P-70

TARGETED ANTICANCER THERAPY OF POLYMER CONJUGATES TO BREAST CANCER

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Multivalent polymeric systems are an ideal platform for a 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 anti-angiogenic properties and synergistic cytotoxic effects on endothelial cells and on cancer cells, such as the human breast cancer cell line, MDA-MB-231, and the murine mammary carcinoma cell line, 4T1. A water-soluble polymer-conjugated with those drugs can accumulate in tumors by passive targeting via the enhanced permeability and retention (EPR) effect (*2*). A well-studied polymer is the polyglutamic acid (PGA), which is biocompatible, non-immunogenic, non-toxic, FDA-approved and targetable carrier to which the drugs are bound covalently via a peptidyl spacer. Moreover, PGA is biodegradable by cysteine proteases, particularly cathepsin B (*3*).

We developed a new strategy of combination therapy for the treatment of breast cancer. We conjugated PTX and DOX with PGA; PTX was bound directly to the PGA backbone through the $^{\gamma}$COOH groups of the glutamic acid. DOX was coupled to the polymer backbone via the tetra-peptidyl Gly-Phe-Leu-Gly linker, cleaved by cathepsin B. PGA-PTX-DOX nano-conjugate inhibited the proliferation of both endothelial cells and breast cancer cells. Furthermore, our conjugate demonstrated anti-angiogenic properties. Our preliminary results with PGA-PTX-DOX nano-conjugate present its potential use as a novel anti-angiogenic therapy for breast cancer.

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Keywords: combination therapy, polyglutamic acid, nano-conjugate

P-71

QUANTITATIVE STRUCTURE – PROPERTY RELATIONSHIP MODELING OF REMOTE LIPOSOME LOADING OF DRUGS

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Remote loading of liposomes by trans-membrane gradients is used to achieve therapeutically efficacious intra-liposome concentrations of drugs. We have developed Quantitative Structure Property Relationship (QSPR) models of remote liposome loading for a dataset including 60 drugs studied in 366 loading experiments internally or elsewhere. Both experimental conditions and computed chemical descriptors were employed as independent variables to predict the initial drug/lipid ratio (D/L) required to achieve high loading efficiency. Both binary (to distinguish high vs. low initial D/L) and continuous (to predict real D/L value) models were generated using advanced datamining methods and five-fold external validation. The external prediction accuracy for binary models was as high as 91-96%; for continuous models the mean regression coefficient R^2 for correlation between predicted versus observed values was 0.76-0.79. This novel approach can be used to identify candidate drugs expected to have high remote loading capacity while simultaneously optimizing the design of formulation experiments.

Keywords: chemical deors; liposome; loading conditions; loading efficiency; QSPR; remote loading

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STERICALLY STABILIZIED NANO-LIPOSOMES HAVING favorable PHARMACOKINETICS AND CONTROLLED DRUG RELEASE RATE FOR MEDICAL APPLICATIONS

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Twenty years ago the use of sterically stabilized nano-liposomes (nSSL) for intravenous drug delivery was introduced. The first such product approved by the FDA was the anticancer nano-medicine Doxil™, developed in our laboratory. Our current research is focused on the use of nanotechnology to improve nSSL performance as a drug delivery system to treat diseases such as cancer and diseases having an inflammatory component, including multiple sclerosis (MS), rheumatoid arthritis (RA) and amyotrophic lateral sclerosis. For this we are developing two different liposomal drugs: (1) liposomal glucocorticosteroids (nSSL-GCs) used systemically and subcutaneously as anti-inflammatory and anti-autoimmune therapy, and (2) a liposomal antioxidant, tempamine (nSSL-TMN). We prepared ~80-nm pegylated nano-liposomes remote loaded with the "water-soluble" amphipathic weak acid steroid prodrugs methylprednisolone hemisuccinate sodium salt and betamethasone hemisuccinate sodium salt, or the amphipathic weak base nitroxide antioxidant tempamine. Our results from 2 different murine models: experimental autoimmune encephalomyelitis, an accepted animal model for the neurodegenerative disease MS and adjuvant-induced arthritis, an accepted model for RA, clearly show that these formulations have therapeutic efficacy much superior to the free drugs and to most drugs currently used to treat these diseases. These nSSL selectively accumulate at sites of enhanced vascular permeability such as inflamed tissues. Accumulation of drug-loaded nSSL at these sites, followed by drug release there, explains the superior therapeutic efficacy of these nanomedicines.

Keywords: nano-liposmes, Inflammation

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

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Polymeric nano-carriers conjugated to low molecular weight drugs are used for improving the efficacy and toxicity profile of medicines. In particular, this approach is beneficial for antitumor drugs, where the polymer-drug conjugates accumulate at the tumor site, due to the enhanced permeability and retention (EPR) effect. Usually, the conjugated drug is inactive, and upon its release (pH or enzymatic) from the polymer it regains its therapeutic activity. However, data on time and location of drug release from the polymeric nano-carriers*in vivo*is lacking. Real-time non-invasive monitoring of the drug release process is desirable for using such reporting nanomedicines in theranostics (therapy and diagnostics)[1][2].

In this study, we coupled a fluorophore to a polymeric nanomedicine. Thus, real-time information about the release process can be obtained optically using non-invasive fluorescence detection techniques. Near-infrared (NIR) fluorophores, such as Cy5 and Cy7, allow detection through deep tissues imitating the clinical setting where tumors are inoculated orthotopically. When conjugated to a polymer at a suitable percent loading, the fluorophores are self-quenched. Upon enzymatic degradation, the release of the chemotherapeutic drugs will concomitantly occur with the release and activation of the fluorophore to its TURN ON state.

We are currently evaluating two systems of polymer-drug-fluorophore conjugates, as well as different methods for quenching. After developing a working system in cells, we will use it to characterize the release process of the drug from the nano-carrier in tumor-bearing mice.

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Keywords: Theranostics; NIR fluorophores; Polymeric nanocarriers

P-74

IODINE RELEASE FROM POLYURETHANE SPONGES FOR WATER MICROBIAL DISINFECTION

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Iodinated polyurethanes sponges (IPU) were prepared by immersing sponges in aqueous solutions of iodine or iodine vapors. These Iodinated PU sponge were coated with pEVA (CIPU) for potential use as antimicrobial slow release agent for water purification systems combined with active carbon cartridge. Iodine was readily absorbed up to 100% w/w in the polymers. Iodine was release for over 250 litter of water passing through the sponge as determined by iodine analysis and monitoring the antimicrobial activity using E. coli and MS2 cultures. EVA coated PU sponges exhibited marked antifungal and antibacterial activities against the fungal/bacterial strains tested. Bacterial results exceeded the minimal requirement for bacterial removal of 6 log reduction throughout the entire life – span. At any testing point, no bacteria was detected in the outlet achieving more than 7.1 to more than 8 log reduction as calculated upon the inlet concentration. Virus Surrogate, MS2, reduction results varied from 5.11 log reduction under basic water (TW2 pH 9) to 1.32 for acidic water (TW2 pH -5). Iodine absorbed in polyurethane sponges are effective is deactivating bacteria and viruses in water and thus can be incorporated in water purification filters.

Keywords: Iodine polyurethane antimicrobial

P-75

REVERTING THE FAST-GROWING PHENOTYPE OF HUMAN GLIOBLASTOMA USING AN ANTI-ANGIOGENIC THROMBOSPONDIN MIMETIC PEPTIDE

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Small sized, microscopic, avascular and therefore asymptomatic tumors can remain in their dormant stage for a considerable period of time depending on numerous processes. One crucial mechanism underlying the transformation from a dormant to a fast-growing phenotype is the ability of tumor cells to induce angiogenesis, a phenomenon termed as the "angiogenic switch". We have identified and isolated a dormant tumor-generating clone, derived from the aggressive tumor-forming U-87 MG human glioblastoma cell line (1), using gene expression signature of dormant tumors (2). The two cell lines exhibit profound differences in their angiogenic potential and gene expression involved in angiogenesis regulation. One of the major dissimilarities was found in thrombospondin-1 (TSP-1) expression levels. The dormant avascular tumor-generating cell line (U-87-D) express significantly higher levels of TSP-1 compared to the fast-growing angiogenic tumor-generating parental cell line (U-87-F). It has been previously demonstrated that TSP-1 is a key endogenous angiogenesis inhibitor. Therefore, it has been established as an attractive potential therapy for angiogenesis-dependent diseases.

In this study, we evaluated the ability of TSP-1-peptidomimetic to regress the fast-growing angiogenic phenotype of U-87-F to the dormant avascular phenotype of U-87-D. Mice bearing established U-87-F tumors received daily treatment (50 mg/kg/day), by intra-peritoneal injection concomitantly by slow-release ALZET® osmotic pump (0.5 μl/h). TSP-1-peptidomimetic attenuated tumor progression in treated mice compared with control mice. Immunohistochemistry analysis of treated tumors revealed reduced abnormal vasculature, increased αSMA expression and decreased VEGF expression. We concluded that TSP-1-peptidomimetic in combination with chemotherapy may present a promising treatment for progressive glioblastoma.

Keywords: Tumor dormancy, the angiogenic switch, TSP-1 mimetic

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P-76

ENHANCED PERCUTANEOUS PERMEATION OF DEHYDROEPIANDOSTERONE-LOADED NANOCAPSULES

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Skin permeation of poorly-absorbed active ingredients can be improved by adding specific enhancers to the formulation, or using nanodelivery systems, especially nanoparticles (NPs) or nanocapsules (NCs). The endogenous hormone dehydroepiandrosterone (DHEA) was reported to decrease with aging and is known to precipitate in topical formulations owing to its complex solubility in common solvents resulting in limited skin absorption. The objective of the present study is to design a PLGA-based nanocarrier, free of DHEA crystals, in an attempt to enhance the skin permeation of this steroid and elicit beneficial local anti-aging effects. DHEA loaded NCs, containing different oils, were prepared using the solvent displacement method. In the absence of oils, NPs were formed. The *ex–vivo* penetration of DHEA on excised human skin using various radioactive [^{3}H]DHEA -loaded NCs and respective controls was examined. DHEA loaded NPs and NCs exhibited mean diameters of 80 and 180 nm and a zeta potential of -30 mV, respectively. DHEA 'crystals free' preparation was obtained only when NCs had formed. MTT assay results using HaCat cell culture showed that the mid-chain triglycerides (MCT) containing NCs remained non-toxic in polymer concentrations up to 3mg/mL. Increasing levels of the radioactive DHEA were recorded over time in the viable skin layers when DHEA-loaded NCs were incubated, while the respective oil controls exhibited lower [^{3}H]DHEA levels. These overall findings suggest that the utilization of adequate and biodegradable polymeric nanocarriers can enhance the percutaneous permeation of DHEA.

Keywords: Skin permeation, DHEA, nanocarrier

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TARGETED GENE DELIVERY INTO ANTIGEN-PRESENTING CELLS USING POLYION COMPLEXES (PIC)

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Introduction: Dendritic cells (DC) are the most potent antigen-presenting cells. Due to their unique properties in initiating primary immune responses, they are an excellent target for the delivery of DNA/RNA based activation. We constructed a non-viral gene delivery vehicle consisting of poly(ethylene imine) (PEI), poly(ethylene glycol) (PEG) and carbohydrate or non-carbohydrate targeting moiety. PEI serves as a binding, protective and transfection-inducing unit for the negative DNA/RNA. The PEG unit can reduce opsonization and thus prolong the circulation time in the bloodstream. Mannose (Man) and mannose mimetic (Quinic acid, Qa) were selected as targeting ligands for the mannose receptor (ManR) on dendritic cells. The block copolymers can produce a complex with a micelle-like structure around the DNA/RNA and can enter DCs using the ManR uptake route.

Methods: A mono-Man, tri-Man, mono-Qa and tetra-Qa ligands were constructed. The ligands were conjugated to the PEG and PEI polymers, to form targeted-PEG-PEI copolymers. ^{1}HNMR and MALDI-TOF analysis confirmed the structure of the desired products. Complex formation with DNA/RNA was determined by Ethidium bromide assay. The surface charge was determined by ζ-potential and the transfection efficacy was determined using luciferase activity assay on DC cells.

Results and Discussion: Block copolymers bearing the different targeting ligands showed complexation with DNA/RNA at N/P ratios > 4, and had lower surface charge relative to their PEI building block. Man_3-PEG--PEI demonstrated greater transfection efficiency relative to Man-PEG--PEI. The mono- and tetra-Qa ligands are currently being investigated for their transfection efficiency.

Keywords: Dendritic cells, Gene delivery, PIC

P-78

MAGNETIC SCAFFOLDS ENRICHED WITH BIOACTIVE NANOPARTICLES FOR TISSUE ENGINEERING

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Effective biological scaffolds for tissue engineering and regenerative medicine applications are constantly being developed.[1] These materials emerge as fundamental tools to help the body rebuild damaged or diseased tissues due to their ability to provide a supporting three-dimension (3D) environment for specific cell populations, guiding their growth and function. In the present study, we report on the fabrication of novel magnetic fibrin hydrogel scaffolds for cell implantation and tissue engineering.[2] The magnetic scaffolds were produced by the interaction of thrombin conjugated iron oxide (maghemite, g-Fe_2O_3) nanoparticles of 23 ± 4.7 nm diameter and fibrinogen. The fibrin viscous solution, before gelation, was subcutaneously injected into adult rats' thighs, and the formed scaffolds were then visualized by magnetic resonance imaging (MRI). In order to enhance cell regeneration, the magnetic scaffolds were enriched with growth factors, e.g., basic fibroblast growth factor (bFGF), beta nerve growth factor (βNGF) or glial cell-derived neurotrophic factor (GDNF) by covalent conjugation to fluorescent rhodamine g-Fe_2O_3 nanoparticles (R-g-Fe_2O_3). These scaffolds enriched with the bioactive nanoparticles provide a highly efficient supporting environment for massive 3D growth and proliferation of various cells, e.g., nasal olfactory mucosa (NOM) and dorsal root ganglia (DRG) cells. These studies also indicated for the first time that the bFGF conjugated nanoparticles, either covalently or physically, significantly improved the growth of NOM cells seeded in the magnetic scaffolds, as compared to the same concentration, or even five times higher, of the free factor.[3]

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Keywords: Fibrin hydrogel scaffolds, Iron oxide nanoparticles, Tissue engineering

P-79

REGRESSING THE ANGIOGENIC SWITCH OF CALCIFIED NEOPLASMS USING COMBINED POLYMER THERAPEUTICS

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Angiogenesis is a key process in tumor progression and metastases[1]. Therefore, we developed a new therapeutic strategy to target bone metastases and calcified neoplasms using combined polymer-bound angiogenesis inhibitors[2]. Using the reversible addition-fragmentation chain transfer (RAFT) technique, we conjugated the aminobisphosphonate alendronate (ALN), and the potent anti-angiogenic agent TNP-470 with N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer through a linker, cleaved by cathepsin K, overexpressed at resorption sites in bone tissues[2]. We show that free and conjugated ALN-TNP-470 have synergistic anti-angiogenic and antitumor activity by inhibiting the *in vitro* proliferation, migration and capillary-like tube formation of endothelial and human osteosarcoma cells.*In vivo,*our conjugate reduced VEGF-induced vascular hyperpermeability by 92% and remarkably inhibited osteosarcoma growth in mice by 96%. In order to imitate the clinical setting, in which tumor progression depends on sequential events, including a switch to the angiogenic phenotype, (i.e., initial recruitment of new blood vessels), we developed a model of tumor dormancy based on Saos-2 human osteosarcoma cells. We are currently evaluating the ability of our conjugate to delay the angiogenic switch and keep the dormant tumors avascular for longer periods or alternatively to regress the fast-growing angiogenic tumor to a dormant phenotype. The new therapeutic platform described here may have clinical utility as a potential therapy for patients with primary osteosarcoma and for those with high risk of outbreak of bone metastases originating from prostate and breast cancers.

Keywords: Angiogenesis, Metastases, Conjugate, Dormancy, Therapy

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P-80

ORGANIC NANOPARTICLES FROM MICROEMULSIONS: FORMATION AND APPLICATIONS

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Hydrophobic substances and drugs can be transformed into freely water-dispersible nanoparticles by a simple process based on a rapid conversion of oil-in-water microemulsion containing the insoluble substance into dry powder composed of nanoparticles. The nanoparticles are obtained from the spontaneously formed microemulsions, which are comprised of nanometric droplets of volatile oil with the dissolved active ingredient. Rapid simultaneous evaporation of the oil and the water leads to a conversion of the droplets into nanoparticles, in the form of a dry powder. This powder is easily dispersible in water, biological fluids and other aqueous media. It was found that nanoparticles in powders prepared by this method exhibit much better dissolution properties and improved biological activity, as compared with the bulk material. Good examples are nanoparticles of celecoxib and simvastatin, prepared by this method. Often the final size of the obtained particles is larger than the size of the initial microemulsion droplets. When the initial system was formed by a single catanionic surfactant, the final particles were smaller then the initial droplets, attesting to the probable preservation of the microemulsion structure and higher rigidity of the interface. Using bile salt derivative as the surfactant for microemulsion enabled a significantly higher loading of the celecoxib in both microemulsion and nanoparticles. In addition, a superior stability of the particles formed with the bile salt derivative and its crystallization inhibition properties were observed. Furthermore, after dispersing the nanoparticles in water, they spontaneously arranged into well-defined elongated nanometric tubules.

Keywords: Nanoparticles, microemulsions, crystallization

P-81

PREDICTING MICRORNA EXPRESSION PATTERNS INVOLVED IN TUMOR DORMANCY USING ANTI-ANGIOGENIC NANOMEDICINES

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There is an increasing interest in elucidating the mechanisms at which dormant tumors acquire the ability to grow and metastasize. Although the tumor dormancy phenomenon has important implications for early detection and treatment of cancer, its biology and genetic characteristics are poorly understood. We recently reported of prolonged dormancy period of fast-growing osteosarcoma in response to treatment with our novel anti-angiogenic HPMA copolymer-alendronate-TNP-470 conjugate. We set to explore the molecular pathways leading to this prolonged dormancy period in order to shed light on the mechanisms which govern the angiogenic switch. We therefore established a pair of cell lines that generate dormant avascular and fast-growing angiogenic osteosarcomas in SCID mice (Saos-2-D and Saos-2-E respectively). Following our hypothesis, microRNA (miR) array of Saos-2-D and Saos-2-E cells, either treated with the conjugate or untreated, was performed. We focused on miRs which signature is changed from a fast-growing to a dormant phenotype or *vice versa* by treatment with our conjugate. Those miRs were overexpressed and consequent phenotype changes were monitored *in vitro* and *in vivo*, compared with the parental cell lines. Overexpression of miR-200c in Saos-2-E cells resulted in regression to the dormant phenotype both *in vitro* and *in vivo.* We propose that miRNA-200c may play a key role in the angiogenic switch and may be used for osteosarcoma therapy in combination with an appropriate delivery system. This data suggests that our polymer therapeutics may be used as a pharmacological tool for prediction of miRs associated with the switch from dormancy.

Keywords: Tumor dormancy, Nanomedicines, microRNA

P-82

KNEE POST OPERATIONAL PAIN TREATMENT USING BUPIVACAINE INJECTABLE POLYMER FORMULATION

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Background: Following orthopedic operation, pain induced can interfere with the social life and rehabilitation of the patient producing feelings of discomfort and hopelessness. Use of local anesthetics may have a significant role in future postoperative pain management in knee arthoplasty, and therefore evaluation of the optimal effect of the administrated local anesthetics should be done. The purpose of this study was to determine if an intra-operative intra-articular injection of poly(lactic acid co castor oil3:7), DLLA:CO loaded with 15% bupivacaine, a local anesthetic, can be useful for postoperative pain relieve after knee arthroplasty.

Methods: The polymer was synthesized by ring opening polymerization of castor oil and DL-Lactide. The final polymer was loaded with 15% bupivacaine. a single injection of 0.2mL of 15% polymer–bupivacaine formulation or plain drug was injected through in the knee joint area following knee arthroplasty in a rat model and compared to a non surgery control group. Under anesthesia rats knee junction was exposed and a hole drilled near the joint. Behavioral tests including functional assessment of the joint and mechanical hypersensitivity were performed for four days following the operation.

Results: All animals recovered well from surgery. Changes in behavior like reduction in motor activities in the treated groups were observed following the operation. The control group expressed higher activity during the evaluation period. Similar results in pain assessment were obtained for the bupivacaine loaded polymer formulation and the control group during the first 48h following surgery, which indicates that the formulation was efficient for pain control during this period of time compared with the commercial plain drug that was effective for less than 24h.

Conclusion: Analysis of the data evidenced that bupivacaine encapsulated in poly (DL-lactic acid co castor oil) 3:7 extended the duration of the analgesia on the treated rats and could represent and effective postoperative analgesic in orthopedic procedures.

P-83

INCREASED CELL INTERNALIZATION BY CONVECTION ENHANCED DELIVERY OF LIPOSOMES

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Convection-enhanced drug delivery (CED), by creating an infusion-mediated pressure gradient through intracranial catheters, greatly enhances the distribution of drugs in the brain. This technique (currently in advanced clinical trials) enables in situ drug concentrations several orders of magnitude greater than those achieved by systemic administration over large brain volumes. We have previously demonstrated the feasibility of adding free Gd-DTPA to the convected infusate for real-time depiction of the drug distribution in the target tissue. Since Gd-based contrast agents do not penetrate the cells, follow-up MRI performed 24 hours post treatment shows no residual Gd in the brain.

In the current study we assessed the feasibility of using PEGylated and non-PEGylated liposomes to obtain increased cell internalization of therapeutic agents delivered by CED. Gd-DTPA was encapsulated in 150nm liposomes. MRI evaluation of in vitro experiments showed that Gd-DTPA was effectively internalized by CNS-1 rat glioma cells 24 hr after incubation with both types of liposomes. MRI evaluation of rats treated by intracranial CED of Gd-DTPA in saline showed no residual Gd-DTPA 48 hours post treatment. Rat treated by CED of liposomes showed significant enhancement in the treated region up to 2 weeks post treatment, suggesting significant Gd-DTPA internalization in the rat striatum. The distribution volume of the PEGylated liposomes in the brain was nearly twice than that of the non-PEGylated liposomes (35.2±4.2 vs 15.4±2.0 mcl, $P<0.001$). In ongoing studies we evaluate Gd/drug formulations in glioblastoma multiforma (GBM) animals for enhanced detection by MRI and a therapeutic effect.

P-84

TARGETING BREAST CANCER BONE METASTASES USING POLYMER THERAPEUTICS BEARING PACLITAXEL AND ALENDRONATE

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Bone metastases are one of the main obstacles for the recuperation of breast cancer patients. Therapy includes the taxane, paclitaxel (PTX), and the bisphosphonate, alendronate (ALN). There is growing evidence that low-dose taxanes and bisphosphonates posses anti-angiogenic properties. However, PTX is water insoluble and toxic, even if administered at low anti-angiogenic concentrations.

Polymer therapeutics could be used to overcome the pharmacokinetics and biodistribution limitations associated with PTX: It can increase the drug water-solubility, prolong its circulation time, achieve its controlled and sustained release, and enhance its selective accumulation in tumor sites due to the enhanced permeability and retention (EPR) effect. Furthermore, targeting agents such as ALN could also be combined in polymer-drug conjugates.

We designed and synthesized two polymer conjugates of PTX and ALN. In the first conjugate, an N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-PTX-ALN, PTX was bound to HPMA copolymer-Gly-Phe-Leu-Gly-p-nitrophenol (HPMA copolymer-GFLG-ONp) through Phe-Lys-p-aminobenzyl carbonate (FK-PABC) spacer. This spacer is cleaved by the lysosomal enzyme cathepsin B, which is overexpressed in tumor tissue. The additional polymer therapeutic consists of ALN, and the chemotherapeutic agent PTX conjugated with poly(ethyleneglycol) (PEG) bearing a β-Glutamic acid dendron at one end of the polymeric backbone. PTX was conjugated to PEG by an ester bond and four molecules of ALN were conjugated through the β-Glutamic acid dendron. The PEGylated PTX-ALN conjugates were designed to self-assemble and form micelles in which PTX is at the hydrophobic core, whereas ALN is at the hydrophilic shell.

We have found that both PTX-PEG-ALN and HPMA copolymer-PTX-ALN are water soluble, posses high affinity to the bone mineral hydroxyapatite, and exhibited antitumor and anti-angiogenic efficacy. PTX-PEG-ALN has also demonstrated prolonged blood circulation and selective tumor accumulation.

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P-85

THE INTERACTIONS OF POLYMERIC NANOCARRIERS WITH CELL MONOLAYER AND EXCISED HUMAN SKIN

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Although nanoparticles (NPs) and nanocapsules (NCs) can promote the dermal diffusion of their cargo, their skin biofate is still not completely elucidated. This study evaluates the interactions of these nanocarriers with cell monolayer and their respective internalization using excised human skin. NPs and NCs were prepared and envisioned using TEM. Double fluorescent labeled NPs and NCs were prepared with PLGA that was covalently conjugated to the rhodamine B using carbodiimide chemistry and confirmed using FTIR spectroscopy, ^{1}H-NMR and Gel Permeation Chromatography. In addition, the fluorescent probe DiD was loaded within MCT NCs and NPs. NPs and NCs exhibited mean diameters of 95 and 180 nm, respectively and a zeta potential of -26 mV. MTT assay results using HaCat cell culture showed that both fluorescent and conventional nanocarriers remained non-toxic in polymer concentrations up to 3mg/ml. Time and concentration depended cellular uptake of the various dually labeled NPs and NCs showed encouraging results, suggesting that both fluorescent probes penetrated the cells following endocytosis of the nanoparticulate delivery systems. Site-localization experiments were performed on excised human skin in Franz diffusion cells using confocal microscopy fluorescent imaging. Vertical skin sections and 3D images of whole treated skin samples reconstructed from z-stacks of the NPs and NCs following 2h post topical application showed that more of the cargo was released from NCs than NPs although both reached the same depth close to 200 μm while the respective controls remained on the superficial skin layers.

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