THE 7 TH ANNUAL MEETING OF THE ISRAELI CHAPTER OF THE CONTROLLED RELEASE SOCIETY (ICRS)

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

October 3-4, 2010

Dan Carmel Hotel, Haifa

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TRANSPHARMA MEDICAL

PROGRAM

Sunday, October 3, 2010

Monday, October 4, 2010

Sunday –October 3, 2010

8:30 - 9:00 **REGISTRATION AND POSTERS SETUP**

9:00 - 9:10 **Welcome**
Rosa Azhari, ICRS *President*

9:10- 9:55 **KEYNOTE PRESENTATION**
SMART AND DRUG-FREE MACROMOLECULAR THERAPEUTICS
Jindrich Kopecek
Department of Pharmaceutics and Pharmaceutical Chemistry, Department of Bioengineering, University of Utah, Salt Lake City, Utah, USA

10:00 - 12:50 **SESSION 1**
NOVEL ANTI-CANCER THERAPIES
Chairpersons**:** ***Alberto Gabizon and Jindrich Kopecek***

10:00 – 10:25 **Delivery of Anticancer Agents in Liposomes: From Bench to Bedside**
Alberto Gabizon
Shaare Zedek Hospital and The Hebrew University of Jerusalem

10:25- 10:50 **Rational Design of Multifunctional Polymer Therapeutics for Cancer Theranostics**
Ronit Satchi-Fainaro
Department of Physiology-Pharmacology, Sackler School of Medicine, Tel Aviv University

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

11:10- 11:35 **Novel Polymer Conjugates for Cancer Detection and Therapy**
Ayelet David
Department of Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev

11:35- 12:00 **Intracellularly-Targeted Drug Delivery**
David Stepansky
Department of Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev

12:00- 12:30 ***Short presentations of selected posters:***

Maria Popov, *Delivery of analgesic peptides to the brain by nano-sized vesicles made of monolayered membranes*

Alina Shapira, *Beta-Casein micelles as oral nano-vehicles for chemotherapeutic drugs*

Adi Tzur, *Surface engineered porous silicon-based nanostructures for cancer therapy*

Ektarina Perets, *Controlled delivery system to elicit anti-cancer effects of HIV protease inhibitor Nelfinavir*

Paula Ofek, *In-vivo delivery of SIRNA to tumors and their vasculature by novel dendritic nanocarriers*

Yosi Shamay, *E-Selectin targeted polymer conjugates for the treatment of primary tumors and lung metastasis*

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

13:30- 14:15 **KEYNOTE PRESENTATION**

SUPRAMOLECULAR BIOCONJUGATES FOR DRUG DELIVERY

Paolo Caliceti

Department of Pharmaceutical Sciences, University of Padova, Padoua, Italy

14:15-15:30 **SESSION 2**

IMAGING, DIAGNOSTICS AND PRUDUCT CHARACTERIZATION IN ADVANCED DRUG THERAPIES

Chairpersons: ***Avri Rubimstein***

14:15-14:40 **Non-Invasive Cancer Imaging Using Near Infrared Fluorescent Cathepsin Activity-Based Probes**

Galia Blum

Institute of Drug Research, Hebrew University of Jerusalem

14:40-15:05 **Distinctive Chemical Approach for Real-Time Imaging of Drug Release**

Doron Shabat

School of Chemistry, Tel Aviv University

15:05-15:25 **Advanced Cryo-Electron Microscopy in the Study of Nano-Aggregates in Liquid and Semi-Liquid Systems**

Yeshayahu Talmon

Faculty of Chemical Engineering, Technion-Israel Institute of Technology, Haifa

15:25-15:45 **Immunosensing Platforms for optical Detection of Cancer Biomarkers in the Lumen of the GI Tract**

Elena Khazanov

School pf Pharmacy, Institute for Drug research, The Hebrew University of Jerusalem

15:45-15:55 ***Short presentations of selected posters:***

Arik Zur, *Comparison between the pharmacokinetic properties of two non-metabolic long chain fatty acid analogs for the therapy of metabolic syndrome*

Avital haft, *Reduction of chemical bonds by bacteria of the colon*

15:55- 17:00 **POSTER SESSION AND COFFEE**

17:00-18:45 **SESSION 3**
BIOAVAILABILITY AND ORAL DELIVERY
Chairpersons: ***Amnon Hoffman and Paolo Caliceti***

17:00-17:25 **A Novel Trojan Nanocarrier Enhances Markedly the Oral Docetaxel Absorption via the Lymphatic Route**
Simon Benita
Institute for Drug Research, The Hebrew University of Jerusalem

17:25-17:50 **Characterization of the Intestinal Drug Absorption Mechanism for Optimizing Oral Delivery**
Amnon Hoffman
Institute for Drug Research, The Hebrew University of Jerusalem

17:50-18:15 **Starch Particles for Enhanced Bioavailability of Edible Bioactives**
Eyal Shimoni
Faculty of Biotechnology and Food Engineering, Technion, Haifa

18:15-18:40 **Gastro intestinal Tracking of Enterically Coated Capsules Using X-Ray Imaging**
Sigal Saphier
Department of Organic Chemistry, Israel Institute for Biological Research, Nes Ziona

20:30 **GALA DINNER**

- **Presentation of the ICRS Prize for Outstanding Achievements in Drug Delivery**

- **Presentation of the 2009 Best Student Poster Awards:**
  First prize:
  Daniel Zucker, Cancer therapeutic efficacy of two drug combination co-remote loaded into nanoliposomes: relevance of in-vitro synergy, Hebrew University of Jerusalem. Advisor: Prof. Chezy Barenholz.
  Second prize:
  Ehud Segal, RAFT- Synthesized nanoconjugates for targeting bone metastases and calcified neoplasms, Tel Aviv University. Advisor: Dr. Ronit Satchi-Fainaro.
  Third prizes:
  Emil Rubinov, Affinity-binding alginate biomaterial for the controlled delivery of cardiovascular-protective factors, Ben-Gurion University of the Negev. Advisor: Prof. Smadar Cohen.
  Eva Kopansky, Polymer conjugates for visualizing solid tumors in the GI tract, Ben-Gurion University of the Negev. Advisor: Dr. Ayelet David.
  Lior Raviv Mannosylated block copolymer micelles for targeting genes into antigen-presenting cells, Ben-Gurion University of the Negev. Advisor: Dr. Ayelet David.

- **Announcement of the 2010 Best Student Presentation Award Winners 2010 Award sponsored by Professor Chezy Barenholz**

Monday –October 4, 2010

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

8:30 - 9:15 **KEYNOTE PRESENTATION**
TARGETING THE IMMUNE SYSTEM TO CANCER
Alexander Levitzki
Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem

9:15-10:55 **SESSION 4**
ISSUES IN INFLAMMATION AND IMMUNOMODULATION
Chairpersons**:** ***Rimona Margalit and Alexander Levitzki***

9:15-9:40 **Immuno-modulation by Nanoparticles in Cardiovascular Disorders**
Gershon Golomb
Departrment of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem

9:40-10:05 **RNAi Nanomedicines : Challenges and Opportunities within the Immune System**
Dan Peer
Department of Cell Research and Immunology, Faculty of Life Sciences, Tel Aviv University

10:05-10:30 **Novel Fibrillar Insulin Formulations for Oral Administration**
Yaron Dekel
Migal Institute, Kiryat Shmona

10:30-10:55 **Targeting Transferrin Receptor with PEGylated Nano-Immunoliposomes: a Potential Method for the Local Treatment of IBD via the Luminal Route**
Efrat Harel
Institute for Drug Research, The Hebrew University of Jerusalem

10: 55-11:15 **COFFEE BREAK**

11:15-13:30 ***SESSION 5***
DRUG DELIVERY : FROM RESEARCH TO COMMERCIAL PRODUCTS
Chairpersons: ***Joseph Kost and Chezy Barenholz***

11:15- 12:00 **KEYNOTE PRESENTATION**
A FRESH START(UP), THE LIFE CYCLE OF A START-UP - AN ENTREPRENEUR'S PERSPECTIVE, AN INVESTOR'S VIEW.
Avi Molcho
Venture Partner, Forbion Capital Partners; Founder, Biolojic Design

12:00-12:25 **Patent Protection for Innovations in Controlled Release Drug Technology**
Joseph Wyse
Eyal Bressler & Co. Patent Attorneys

12:25-12:50 **Eliminating Needles: Therapeutic Delivery of Peptides and Oligonucleotides Through Skin**
Galit Levin
TransPharma Medical Ltd.

12:50-13:15 **Designing Long Acting Recombinant Proteins: From Bench to Clinics**
Fuad Fares
ModigeneTech and Faculty of Natural Sciences, Haifa University

13:15-13:30 ***Short presentations of selected posters:***

Ariel Gilert, *Immunomodulation using PLGA delivery of short peptides to antigen presenting cells*
Aharon Azaguri, *Effect of ultrasound and chemical penetration enhancers on transport phenomena of chorioamnion membrane*
Yulia Sapir, *Integration of multiple cell-matrix interactions into inert alginate scaffolds for cardiac tissue regeneration*

13:40-14:30 **LUNCH FOR ALL PARTICIPANTS AND POSTER VIEWING**

14:30-16:35 **SESSION 6**
CONTROLLED RELEASE IN TISSUE REGENERATION TECHNOLOGIES
Chairpersons: ***Smadar Cohen and Rosa Azhari***

14:30-14:55 **Local stimulations in 3D for Controlled Differentiation and Organization of Embryonic Stem Cells**
Shulamit Levenberg
Faculty of Biomedical Engineering, Technion- Israel Institute of Technology, Haifa

14:55-15:20 **Novel Antibiotic-Eluting Composite Structures for Wound Healing Applications: In-Vitro and In-Vivo Study**
Maital Zilberman
Biomedical Engineering Department, Tel-Aviv University

15:20-15:45 **Sequential, Multi-Component-Releasing Scaffolds for Tissue Engineering: Fabrication by the Novel Solvent/Non-Solvent Sintering Technology**
Sarit Sivan
Faculty of Biomedical Engineering, Technion- Israel Institue of Technology, Haifa

15:45-16:10 **Cells, Gels, Tissue, and Issues: Novel Hydrogels as Scaffolds Designed with Affinity-Based Drug Delivery**
Liat Oss-Ronen
Faculty of Biomedical Engineering, Technion- Israel Institue of Technology, Haifa

16:10-16:35 **Sequential Delivery of IGF-1 and HGF from Injectable Alginate Biomaterial Promotes Myocardial Repair after Myocardial Infarction**
Emil Rubinov
Department of Biotechnology Engineering, Ben-Gurion University of the Negev

16:35-16:55 **Bioactive Ceramic Scaffold Conjugated PLGA Particles for Bone Tissue Engineering**
Yael Lupu
Faculty of Biotechnology and Food Engineering, Technion- Israel Institute of Technology, Haifa

17:00 **Concluding remarks**
Rosa Azhari

ABSTRACTS

KEYNOTE
LECTURES

SMART AND DRUG-FREE MACROMOLECULAR THERAPEUTICS

Jindřich Henry Kopeček

Department of Pharmaceutics and Pharmaceutical Chemistry/CCCD, Department of Bioengineering, University of Utah, Salt Lake City, Utah 84112, USA

The concept of polymeric nanomedicines was developed to address the lack of specificity of low-molecular weight drugs for malignant cells. The design principles of the first-generation conjugates include: a polymer-drug linker that is stable during transport and able to release the drug in the lysosomal compartment of target cells at a predetermined rate, adequate physicochemical properties of the conjugate (solubility, conformation in the biological environment), and the capability to target the diseased cells or tissue by an active or passive mechanism. Clinical trials of nanomedicines demonstrated reduced side effects, increased therapeutic efficacy, and improved patient compliance. Although the targetability of macromolecular therapeutics to cell surface antigens/receptors is well established, the manipulation of their subcellular fate needs to be studied. Efforts are underway to design, synthesize, and evaluate the second generation of anticancer nanomedicines with enhanced efficiency. The augmented effectiveness of novel conjugates will be derived from: longer intravascular half-life; controlled biodegradability of the main chain; and potential for double targeting – to tumor cells and to a crucial subcellular organelle.

The lecture will discuss recent results in the development of new backbone degradable *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-drug conjugates by combination of controlled (living) radical polymerization and chain extension via alkyne-azide or thiol-ene click reactions. Next, recent advances in cellular and subcellular targeting of HPMA copolymer – drug conjugates will be evaluated.

Finally, a new paradigm in drug delivery will be presented. It is based on the biorecognition of peptide motifs at the cell surface. Two distinct, oppositely charged pentaheptad peptides (CCE and CCK) were designed to create a dimerization motif. Their structure was designed with the goal of achieving an *antiparallel heterodimeric conformation*. The biorecognition of peptide motifs at the cellular surface is able to control the apoptosis of cells. HPMA copolymers grafted with multiple copies of one biorecognition motif (CCK), crosslink non-internalizing, CCEdecorated, receptors on B-cell surfaces, resulting in apoptosis. This is a *new concept, where the biological activity of drug-free macromolecular therapeutics is based on the biorecognition of peptide motifs*.

The research was supported in part by NIH RO1 grants CA51578, CA132831, GM69847, and EB5288.

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TARGETING THE IMMUNE SYSTEM TO CANCER

Alexei Shir and Alexander Levitzki

Unit of Cellular Signaling, Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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Targeted therapy for cancer has gained momentum during the past decade. Two types of agents have been developed: small molecules and antibodies. Both types of agents were found to have limited efficacy since the oncogenic network is complex and cannot be effectively inhibited by blocking a few pathways (1,2). The recognition that hunting down cancer cells and/or reinstating immune surveillance is the key to winning the war on cancer has led to a number of approaches to harness the immune system to fight cancer. A powerful strategy to draft the immune system to the tumor is to tilt the mediators released by the cancer cells to those that attract the NK cells, T cells and macrophages to attack the tumor. How can that be achieved? Since many cancer cells overexpress different types of surface proteins, which internalize upon ligand binding, we devised non-viral "Trojan horses" that home to these proteins and carry a long chain double stranded RNA (dsRNA). Why dsRNA? Because dsRNA evokes a plethora of signal transduction pathways leading to rapid cell death as well as strong by stander effects, affecting neighboring cells that have not swallowed the dsRNA. Remembering that tumor cells are more vulnerable than normal cells to all stresses (3) we hypothesized that we can define a wide enough therapeutic window to affect almost exclusively tumor cells, sparing the normal cells surrounding the tumors. The strategy has the advantage over other immunotherapeutic approaches since both the innate immune system and the adoptive immune system can be activated. Moreover, our strategy can overcome immuno-inhibitory mechanisms developed by many cancers. Here we briefly describe the success of this approach in the treatment of EGFR over-expressing tumors. In this approach, we are not attempting to attenuate the EGFR signaling that has been found to be weak and temporary, utilizing EGFR kinase directed tyrphostins or anti-EGFR antibodies. We have in fact converted the high expression of EGFR in certain tumors into the Achilles heel of the tumor. We utilized EGF guided chemical vectors carrying the synthetic dsRNA PolyIC that home to the EGFR and internalize into the cell. We have proven that this strategy is effective by either local application to EGFR over-expressing tumors (4) and to disseminated EGFR over-expressing tumors (5). In both cases complete eradication of the tumors is achieved with 100% survival.

References

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SUPRAMOLECULAR BIOCONJUGATES FOR DRUG DELIVERY

Paolo Caliceti

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New colloidal drug delivery platforms are designed with the concrete purpose of optimization of the pharmacological performance of bioacitves and the dream to generate ideal therapeutic machineries. As a result, last generation delivery systems are capable of complex functions, which enable sequential overcoming of multiple biobarriers following a certain time/site determined "logic" of events. These nanocarriers enable the use of preexisting drugs by providing longer circulation times, greater tolerability, and site specific delivery; factors that result in better patient outcomes.

Colloidal multifunctional systems are created by chemical or physical assembling of molecular modules with different physicochemical and biological properties that can properly deliver drugs into the disease compartment by exploiting the peculiar environmental, functional and structural properties of disease compartment, namely tissues, cells and subcellular constructs. Accordingly, a variety of supramolecular composites containing targeting agents and environmental sensitive moieties has been investigated to achieve disease site selective drug delivery. The introduction of "smart" modules can enhance the performance of drug cargos that have been developed to deliver high drug amounts in the disease compartment, in particular in tumors where they passively accumulate by EPR.

Oligo- and polysaccharides and gold nanoparticles are typical examples of drug carriers that can be switched from inert platform into "active" drug delivery systems by functionalization with targeting agents and environmental sensitive moieties.

By virtue of their ability to include drugs into the hydrophobic core, CDs have been combined with polymers to deliver passively anticancer and antiviral drugs into disease sites such as tumours. Folic acid functionalized CD-PEG, where the cyclomaltoheptaose acts as a drug "shuttle", PEG confers solubility and flexibility to the targeting moiety, have been found to display in vitro malignant tumour targeting properties.

Polysaccharides have also been demonstrated to be powerful platform for preparation of polymer bioconjugates for drug delivery. Hyaluronic acid and chitosan, for example, have been chemically conjugated with anticancer drugs and targeting agents to endow polymer therapeutics with site specific drug selectivity and increased therapeutic activity. In our studies, pullulan has been properly manipulated to introduce doxorubicin through a pH sensitive linker and folic acid as targeting agent. The derivative has been found to possess interesting potential properties for tumor treatment.

Stimuli sensitive moieties can also be introduced into colloidal systems to tune the drug delivery properties. Either thermal or pH sensitive polymers, for example, have been found to produce smart drug delivery systems with peculiar in vivo performance.

Gold nanoparticles (AuNp) decoration with temperature sensitive polymers can endow colloidal systems with temperature 'switchable' hydrophilic/hydrophobic character, which can be exploited for biomedical, pharmaceutical and biodiagnostic applications.

The AuNp decoration with thermoresponsive polymers makes possible their microenvironmental thermal control and promoted their cell up-take under specific physio-pathologic conditions or by external stimuli. Gold nanoparticles decorated with a co-polymer based on PNIPAm that exhibits LCST close to the physiological one have been

designed considering that thermal abnormalities present in several disease sites, namely in inflamed or cancerous tissue where the local temperature is about 1.5 °C higher as compared to normal tissue. These particles have been found to possess switchable cell uptake properties, which may be exploited for a variety of medical and pharmaceutical purposes.

Site selective targeting may be achieved by designing supramolecular systems that can be obtained by combining gold nanoparticles, stimuli-sensitive polymers and targeting agents. PNIPAM decorated nanoparticles have been associated with targeting molecules, namely ascorbic acid and biotin. These systems have been found to disclose reversibly the targeting functions depending on slight temperature changes.

In conclusion, supramolecular chemistry offers fascinating opportunities to combine functional modules that can endow systems with tailored biopharmaceutical behaviour. Therefore, based on biomedical and biopharmaceutical considerations, colloidal systems with very different physicochemical properties can be properly designed to realize highly performing nanomedicines.

A FRESH START(UP)

Avi Molcho

Venture Partner, Forbion Capital Partners; Founder, Biolojic Design

The life cycle of a start-up - an entrepreneur's perspective, an investor's view.

INVITED LECTURES

Sunday, October 3

DELIVERY OF ANTICANCER AGENTS IN LIPOSOMES: FROM BENCH TO BEDSIDE

Alberto Gabizon

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Despite the advent of new molecular targeted therapies for cancer, most of the currently used anti-tumor agents have problematic toxicities compromising efficacy, and often resulting in life-threatening events. Liposomes can provide effective control of the release rate and of the tissue distribution of many of these agents. These pharmacokinetic changes often have a major pharmacodynamic impact with attenuation of toxic effects and protection of sensitive tissues from dangerous and unwanted drug exposure. Polyethylene-glycol (PEG) coating of liposomes results in inhibition of liposome uptake by the reticulo-endothelial system and significant prolongation of liposome residence time in the blood stream. A hallmark of these long-circulating liposomal drug carriers is their enhanced accumulation in tumors. The mechanism underlying this passive targeting effect is the phenomenon known as enhanced permeability and retention which has been described in a broad variety of experimental tumor types, and appears also to be a relevant phenomenon in human cancer. Developments in drug loading technology have improved the efficiency and stability of drug entrapment in liposomes, particularly with regard to anthracyclines, vinca alkaloids, and camptothecin analogs. An example of liposome formulation with demonstrated clinical added value is pegylated liposomal doxorubicin, which has demonstrated clinically a favorable safety profile with an impressive reduction in cardiac toxicity and proven efficacy against various malignancies and can be considered as the first anti-cancer nanomedicine approved for clinical use. The clinical pharmacokinetic profile of PLD is characterized by slow plasma clearance and small volume of distribution with large differences (~1000-fold) from free doxorubicin. Another approach applicable to liposomal drug delivery combines the concept design of a stable and long-circulating liposome with chemical modification of a drug to form a lipophilic prodrug with strong association to the liposomal bilayer. This is the case of a prodrug of mitomycin-C activated by thiolytic cleavage. Thiolytic cleavage takes place in the tissue micro-environment with negligible activation in plasma thus preventing drug activation and drug leakage in the blood stream. Pegylated liposomal mitomycin-C prodrug was more effective and less toxic than conventional chemotherapy in the treatment of human tumor models of gastrointestinal origin In summary, liposome-based systems offer a vast array of potential applications in the delivery of cancer chemotherapeutic agents which may result in a substantial improvement of the therapeutic index.

Acknowledgement: A. Gabizon is supported by a Professorship award of the Israel Cancer Research Fund.

Keywords: liposomes, cancer, chemotherapy

RATIONAL DESIGN OF MULTIFUNCTIONAL POLYMER THERAPEUTICS FOR CANCER THERANOSTICS

Ronit Satchi-Fainaro

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Introduction

Angiogenesis, new capillary blood vessel growth from pre-existing vasculature, is crucial for tumor progression and metastases [1]. Consequently, the microvascular endothelial cell, recruited by a tumor, has become an important second target in cancer therapy. The successful development of anti-angiogenic therapies for cancer and ocular diseases represents a major medical advance. This validation proves the importance of angiogenesis as a critical "common denominator" pathway in disease. With the recent successful approval and clinical adoption of anti-angiogenic agents, new questions challenge the scientific and clinical dogmas of the angiogenesis field. Although anti-angiogenic agents offer great therapeutic potential, preclinical and clinical studies suggest that these agents, used as monotherapies, will have a delayed onset of activity and may only induce disease stabilization for advanced malignancy.

Drug delivery technologies including novel polymer therapeutics promise to create new combination treatments. Previous work on caplostatin and other polymer-drug conjugates demonstrated that polymer conjugation increases the half-life of low molecular weight drugs, their water-solubility, their tumor accumulation by the enhanced permeability and retention (EPR) effect, and reduces their toxicity. Multi-modality targeted polymer therapeutics that include anti-angiogenic agents and chemotherapeutics offer the potential for improved efficacy and diminished toxicity in the treatment of cancer and other angiogenesis-dependent diseases [2].

Results and Discussion

Our laboratory has recently designed and characterized some novel combined anti-angiogenic and antitumor polymer-drug conjugates that target both the tumor and its microenvironment. We are synthesizing and using a variety of polymers with distinct characteristics such as multivalency, polydispersity, biocompatibility and biodegradability profiles. The polymer-drug conjugates include combined anti-angiogenic and chemotherapeutic drugs, such as TNP-470 [3, 4] and paclitaxel [5], respectively. Some also incorporate bisphosphonates as targeting moieties for bone metastases originating from breast and prostate cancers [5] and osteosarcomas [6] or RGD peptidomimetics [7, 8] that target $\alpha_V\beta_3$ integrins overexpressed on tumor endothelial cells and several tumor cells. Alternatively, the use of siRNA to silence a key regulator gene in tumor progression was utilized using a polyglycerol-amine dendrimer as a selective carrier to the tumor site [9]. The unique chemistry involved with the polymer synthesis and the conjugation with the drugs will be discussed in details. In particular, the conjugation of the aminobisphosphonate alendronate (ALN), and the potent anti-angiogenic agent TNP-470 with *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer using a "living polymerization" technique, the reversible addition-fragmentation chain transfer (RAFT) will be described as an example to a resulting narrowly-dispersed well-characterized polymer therapeutic. Cellular internalization of the conjugates revealed cytoplasmatic uptake by endothelial and tumor cells via endocytosis. The conjugates demonstrated their anti-angiogenic activity by preventing human umbilical vein endothelial cells (HUVEC) from proliferating, migrating towards vascular endothelial growth factor (VEGF) and forming capillary-like tubes in a similar manner as the combination of the free drugs at equivalent concentrations.

Moreover, when anticancer potency was evaluated *in vivo*, the conjugates inhibited the tumor growth rate and their microvasular density.

Our results point at our polymer therapeutics as novel bi-specific conjugates targeting both the tumor epithelial and endothelial compartments warranting their use on a wide spectrum of primary tumors and metastatic ones.

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NOVEL POLYMER CONJUGATES FOR CANCER DETECTION AND THERAPY

Ayelet David

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Over the past few decades, different tumor-targeted drug-delivery systems based on synthetic polymers have been evaluated for improving the therapeutic index of anticancer agents. The conjugation of anticancer drugs and diagnostic agents to biocompatible polymers provides many advantages over small molecular therapeutics, including improved solubility and bioavailability, increased passive accumulation of the drugs and imaging probes at the tumor site by the enhanced permeability and retention (EPR) effect, reduced systemic toxicity of the chemotherapeutic drugs and enhanced therapeutic efficacy. Different combinations of polymer backbones, drug moieties, enzymatic or pH-sensitive spacers for drug attachment, targeting ligands and light-activated peptide sequences have shown superior antitumor efficacy for cancer therapy.

This lecture will describe the design of three novel macromolecular therapeutics based on HPMA copolymers that can (a) actively accumulate at the tumor microenvironment by exploiting targeting moieties for binding to receptor structures expressed on angiogenic blood vessels (b) respond to externally applied light stimuli to promote intracellular drug-penetration (c) facilitate high-resolution in vivo imaging for the rapid, non-invasive monitoring of solid tumors with near-infrared fluorescence (NIRF) probes.

Acknowledgments:

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INTRACELLULARLY-TARGETED DRUG DELIVERY

David Stepensky

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Many biopharmaceuticals act intracellularly and need to reach the site of action in specific organelle to exert their action. Attaining efficient and selective pharmacological effects for these agents requires development of specialized delivery systems (DS) that are targeted to specific organelle and deliver their content in a controlled fashion. For this purpose, particle or vesicle (liposome)-based DS can be used, and intracellular targeting can be achieved by decorating the biopharmaceutical or the DS with organelle-specific targeting moieties.

Feasibility of targeted intracellular delivery of biopharmaceuticals and model compounds has been assessed in several studies. Targeting moieties that were used for this purpose included: 1) peptide sequences that are recognized by the cytosolic transport systems of the host cell, such as nuclear localization signal (NLS), mitochondrial localization signal, endoplasmic reticulum (ER) signal peptide or ER-retrieval sequence, etc.; 2) peptide or non-peptide molecules that preferentially interact with the membrane of the target organelle, e.g. mitochondriotropic arginine-rich peptides or positively charged compounds. However, the quantitative aspects of this targeting, in terms of targeting efficiency and kinetics of delivery of the biopharmaceuticals to the specific intracellular organelles, are not yet clear.

We analyzed the available data on efficiency of different intracellular targeting approaches. It appears that extent of intracellular targeting is dependent on the relative efficiency of two steps. The first limiting step is the ability of the biopharmaceutical or the DS to reach the cell's cytosol. This ability can be enhanced by destabilization of the endosomal membrane using fusogenic liposomes or cell-penetrating peptides, which is potentially toxic to the cells. On the other hand, PLGA nanoparticles can escape from the endo-lysosomes without opening them by mechanism that involves reversal of the PLGA surface charge and direct interaction with membrane. The second limiting step is derived from the interplay of the processes and factors that act on the biopharmaceutical or the DS in the cytosol, i.e., stability and mobility of the targeted agent/DS, efficiency of recognition of the targeting residues by their targets, rate of uptake by the target organelle, etc. The relative efficiency of this step is affected to a high extent by the amount of the targeting residues vs. the size of the agent/DDS.

Based on the analysis of intracellular targeting, we developed approach for enhancing the efficiency of anticancer vaccination based on a novel antigenic peptide-containing DS targeted to the endoplasmic reticulum. We revealed that conjugation with peptide-based targeting moieties affected the intracellular uptake of this DS, its accumulation in the endoplasmic reticulum, and extent of cross-presentation of the antigenic peptide. Effects of this approach on *in vivo* vaccination efficiency will be assessed in the future experiments.

In conclusion, intracellularly-targeted delivery is a promising new approach for enhancing and controlling the pharmacological activities of biopharmaceuticals. Quantitative assessment of the mechanisms and barriers for intracellular delivery will reveal the rate-limiting steps for intracellular targeting of DSs. These outcomes will lead to development of DSs that can efficiently deliver their cargo to the target organelles in the controlled fashion and will enhance the clinical efficiency of the biopharmaceuticals.

NON-INVASIVE CANCER IMAGING USING NEAR INFRARED FLUORESCENT CATHEPSIN ACTIVITY-BASED PROBES

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Cysteine proteases play a pivotal role in normal and pathological processes and have been shown to be highly expressed in active forms during tumorigenesis and other diseases. The cysteine cathepsin proteases therefore serve as good markers for cancer detection. However protease activities are tightly regulated by a series of post-translational mechanisms thereby making simple measurement of protein levels a poor indicator of function. We previously developed fluorescently quenched activity based probes (ABPs) for cysteine proteases that allow real time imaging in living cells as well as non-invasive imaging of protease activity in subcutaneous cancer models. These cell permeable small molecules bind to cathepsin B, L and S through a highly selective enzyme-catalyzed chemical reaction within the active site. This allows direct visualization of cathepsin activity in live cells and in tumors of live mice using simple optical detection methods. Here, we describe an *in vivo* comparison of fluorescently labeled ABPs with commercially available substrate-based probes. Using a Fluorescence Molecular Tomography (FMT) imaging system that detects fluorescent signal deep in the body, we show the advantages of the small molecule probes in terms of the kinetics of labeling, absolute signal, and reduction in background signal compared to the substrate probes. The ABPs are therefore attractive reagents for optical imaging of pathologies deep in the body.

Keywords: Protease Activity, Non-invasive Imaging, Activity Based Probes, Cathepsins, Cancer

DISTINCTIVE CHEMICAL APPROACH FOR REAL-TIME IMAGING OF DRUG RELEASE

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In order to better evaluate the therapeutic effect of drug delivery systems, crucial data on time and location of drug release from the delivery system in living cells and *in vivo*, should be obtained. By coupling latent fluorophore activation to the drug-release event in a delivery system, real-time information about the release process can be obtained using non-invasive fluorescence detection techniques. Light in the near-infrared (NIR) region between 700–900 nm can penetrate deep into living tissue, thereby offering a unique opportunity to use NIR fluorescence imaging to detect and visualize fluorescent probes *in-vivo*. We developed a novel linker with latent fluorescence for assembly of drug-delivery systems. When the system undergoes specific activation, the drug is released and fluorescence is generated through formation of a coumarin derivative. Cathepsin-B-activated prodrug reports its cytotoxic activity toward cancerous cells, by emitting fluorescence. We observed a strong direct correlation between antitumor cell growth inhibition activity and emitted fluorescence. The amount of drug release can be calculated by quantifying the emitted fluorescence; this should allow prediction of a drug delivery systems' therapeutic effect and potential side effects as well as a reporter system for drug release and prodrug activation. Similar probes that based on NIR fluorescence should assist in monitoring cancerous events by utilizing a non-invasive optical imaging device.

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ADVANCED CRYO-ELECTRON MICROSCOPY IN THE STUDY OF NANO-AGGREGATES IN LIQUID AND SEMI-LIQUID SYSTEMS

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Cryogenic-temperature transmission electron microscopy (cryo-TEM) is now accepted as an almost standard tool in the study of complex liquids, i.e., liquid systems with aggregates on the nanometric scale. Methodologies have been developed to help capture the nanostructure of liquid system, while preserving their original state at a given and concentration and temperature. Cryo-TEM is now widely used to study synthetic, biological and medical systems. Originally developed for aqueous systems, it has been also used very successfully in the study of non-aqueous systems.

Recent developments in high-resolution scanning electron microscopy (HR-SEM) have made it an ideal tool for the study of nanoparticles and colloids in viscous systems, or in systems containing large objects (hundreds of nanometers and larger), in which small (nanometric) features are to be imaged. Improved field-emission electron guns, electron optics and detectors have made it possible to image nanoparticles down to a few nanometers. Liquid nanostructured systems can now be studied by cryo-SEM, using much improved specimen preparation equipment and cryogenic specimen holders.

In my lecture I will describe the state-of-the-art of the two techniques, emphasizing possible applications to the characterization of a wide range of nano-aggregates.

IMMUNOSENSING PLATFORMS FOR OPTICAL IMAGING OF CANCER BIOMARKERS IN THE LUMEN OF THE GI TRACT

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Virtual biopsy, based on *in vivo* detection of biomarkers could offer an early detection of malignant processes in the gastrointestinal (GI) tract with the aid of suitable optical machinery such a video capsule[1]. In a previous study we described a diagnostic polymeric platform, capable of detecting α1-antitrypsin precursor (A1AT) in the stomach fluids by immobilized trypsin[2]. In time it was found that the platform suffered from a gradual loss its transparency during modification and detection processes. In this study we describe an improved system comprising of trypsin immobilized to an activated microarray glass slide and a detection reaction generated by fluorescent microspheres (MS) in the near IR (NIR) range.

In a set of screening studies an optimal spacer molecules mix (NHS-^{3k}PEG-NHS with NHS-^{2k}PEG) was re-selected for the attachment of the capturing moiety, trypsin, to the glass surfaces. A specific fluorescent signal (analyzed in a microarray reader), with signal to noise ratio (SNR) of 22-27, dependent on the concentration of the A1AT, was obtained when the system was tested in simulated gastric juice (SGF). However, the SNR was lowered to 4-7 when tested in human gastric juice. The specific fluorescence reaction was, then, tested by an optical capsule simulator (with lower detection capabilities). Due to a low fluorescence signal, generated by the interaction between the Alexa Fluor^{647}-labelled secondary antibody and the captured A1AT, the former was replaced with BODIPY 660-loaded MS onto which the secondary antibody was conjugated. This time the specific interaction between the captured A1AT and the MS could be detected in a biomarker-concentration-dependent manner with a SNR of 6-20. The linearity of this dependency allows a delineation of a working range for the microarray scanner detection in an A1AT concentration range of 10 - 100 μg/ml, with a SNR of 12-32. These findings are currently exploited for the design of a new, real-time diagnostic kit for the early detection of gastric carcinoma.

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A NOVEL TROJAN NANOCARRIER ENHANCES MARKEDLY THE ORAL DOCETAXEL ABSORPTION VIA THE LYMPHATIC ROUTE

Taher Nassar, Suha Attili-Qadri, Oshrat Harush-Frenkel, Shimon Farber, Shimon Lecht, Philip Lazarovici and Simon Benita

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Docetaxel is an important anticancer drug widely prescribed for the treatment of various cancers. Docetaxel administration is associated with the occurrence of unpredictable and acute hypersensitivity reactions attributed, in part, to intrinsic toxic effects of polysorbate 80, a nonionic surfactant needed for the solubilization of the hydrophobic drug in the marketed formulation. Thus, many efforts are being concentrated on the search for alternative docetaxel-free polysorbate formulations with the emphasis on developing oral docetaxel dosage forms. There are several arguments in favor of oral administration, mainly patient convenience, elimination of the risk of infection that is associated with intravenous catheters and marked reduction in hospital admissions. Unfortunately, docetaxel exhibits a low and variable oral bioavailability due to active efflux by P-gp and mainly by CYP3A4 metabolism in the gut wall. We recently proposed a novel oral formulation containing PLGA coated nanocapsules of docetaxel embedded in entero-coated bioadhesive microparticles which were shown to improve the oral bioavailability of docetaxel following the bypass of the gut biochemical barriers. The increased docetaxel overall absorption (AUC) was 158-fold following oral administration of 10 mg/kg dose docetaxel in trojan nanocapsules as compared to the polysorbate docetaxel solution. The level of docetaxel plasma concentrations elicited by the new formulation was even higher than the levels yielded by the intravenous administration of the marketed formulation in rats at the same dose. The absolute bioavailability increased 8.7-fold. These unexpected results could be explained only if the pharmacokinetics and metabolism of docetaxel had been modified. Indeed, we have shown that intact trojan docetaxel nanocapsules do penetrate through the intestinal mucosa, are transported through the lymphatic system and reach the systemic circulation bypassing both gut and hepatic first pass effects. Experiments are being carried out to determine the ratio between free and nanocapsule incorporated docetaxel in plasma. In view of these new findings, there is an opportunity for enhanced accumulation of docetaxel-loaded nanocapsules within the solid tumors owing to the well-known EPR effect. Docetaxel efficacy should be addressed and is currently under investigation.

CHARACTERIZATION OF THE INTESTINAL DRUG ABSORPTION MECHANISM FOR OPTIMIZING THE ORAL DELIVERY SYSTEM

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The oral route is the most preferred mode of drug administration due to its convenience and patient compliance. For many drugs there is a need for a special delivery system to overcome the limited bioavailability following oral administration. In specific cases certain modifications have to be made in the chemistry of the active compound.
In our lab we are developing gastro-retentive dosage forms, based on polymeric blends, that retain in the stomach and release the drug in a controlled manner into the upper part of the intestine. This is done both for extended absorption of drugs characterized by narrow absorption window, and for prolonged activation of luminal receptors within the intestine.
For lipophilic drugs we are developing nanoemulsion formulations that improve both the solubilization as well as presystemic metabolism in the intestinal wall, leading to improved oral bioavailability of problematic and highly variable drugs.
Since peptides are involved in many pathologic conditions, there is a great potential to utilize active peptides as therapeutic agents. However, peptides are degraded rapidly by peptidases in the blood and in the intestinal tract. Using a unique cyclization, named backbone cyclization, we convert active peptide to become metabolically stable. In this approach, by using restricted library of confirmationally diverse analogs, we search for the most active/selective compound that also has the best intestinal permeability properties, thus yielding novel orally available drugs. We are currently utilizing this approach for the treatment of obesity, diabetes, chronic pain, psychiatric disorders, atherosclerosis and AIDS.

Keywords: bioavailability, intestinal absorption, gastroretentive, nanoemulsion, first pass metabolism

STARCH PARTICLES FOR ENHANCED BIOAVAILABILITY OF EDIBLE BIOACTIVES

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The use of food grade biopolymers, such as starch, has been suggested as a technological solution for the controlled delivery of health promoting ingredients. This stable food carbohydrate may form molecular complexes, termed V-amylose, with numerous ligands. Our hypothesis was to use these complexes as a controlled delivery system, which will protect sensitive nutrients during food processing and storage, and will release them in the gastrointestinal tract. In our studies we aimed to develop and asses delivery vehicles for nutrients and bioactive compounds using starch V-complexes.

The ability of these complexes to protect fatty acids from oxidation was first demonstrated with conjugated linoleic acid (CLA). The release of CLA and other guest molecules from the complexes is mostly due to enzymatic digestion by pancreatic amylases, and only small portion is spontaneously released in aqueous solutions. The kinetics of the enzymatic release suggests a slow release process, up to 24 hours, thus suggesting a possible use of these complexes for colonic delivery. Some degree of control over the digestion rate was accomplished by controlling the crystalline polymorphism of the V-complex.
The size of the complexes being formed with various guest molecules (including bulky Isoflavones) can be controlled by their formation method. A continuous dual feed homogenization process, combined with in-situ complexation, as enabled the use of starches from various origins. This methodology yield sub-micron complexes that can then be used as natural nanocapsules for food applications. These sub-micron / nanocapsules are now being applied in staple foods.

We have studies the bioavailability of genistein in amylose-genistein complexes (AGC) in vivo. Bioavailability was tested on Sprague-Dawley rats, placed in metabolic cages, and divided into two groups: one was given high amylose corn starch (HACS) mixed with genistein (AGM) (control), and the second was given AGC. The mean AUC for plasma AGC was significantly higher than that of AGM ($p<0.05$), suggesting that AGC improves genistein bioavailability. Urine results also showed that genistein concentration was significantly higher in the AGC than that of the AGM group. This study demonstrated the ability of the complexes to improve genistein bioavailability.

Keywords: starch, nanoparticles, bioavailability.

GASTRO INTESTINAL TRACKING OF ENTERICALLY COATED CAPSULES USING X-RAY IMAGING.

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The aim of this research was to study the gastrointestinal transit and gastric emptying of enterically coated minicapsules in rats using X-ray imaging. Minicapsules were filled with the X-ray contrast reagent Barium Sulfate and coated with Eudragit S-100. The capsules were administered orally to rats followed by a solution of iodine based contrast agent Iopromide. Thus, the location and integrity of the capsule was easily identified over the background of the highlighted GI tract. Gastric emptying of different sized capsules was studied. The effect of fasting and time of administration on gastric retention was also studied. It was found that 4-5 mm size capsules easily emptied from the stomach whereas 7 mm capsules were retained. Surprisingly, 2.5 hours post administration more capsules were retained in the stomach of rats in the fasted state than in the fed state. Capsules, which remained in the stomach, did not disintegrate for at least 6 hours. The images had very good resolution and the capsule location could be easily identified without special expertise. Capsules were followed through the GI tract from the stomach to the small intestine, cecum and large intestine. Most capsules disintegrated near the end of the small intestine or cecum. Thus, X-ray imaging can be used for simple visualization and localization of solid dosage forms in rats. This method presents a convenient and facile alternative for in vivo imaging of nondisintegrating solid oral dosage forms in rats.

Keywords: X-ray imaging; enteric coating; gastric retention; Rats; Capsules.

INVITED LECTURES

Monday, October 4

IMMUNO-MODULATION BY NANOPARTICLES IN CARDIOVASCULAR DISORDERS

Gershon Golomb

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Intimal hyperplasia is a universal response of the arterial wall to mechanical injury and it is a major cause of restenosis following angioplasty. Experimental and clinical data indicate that the innate immunity and inflammation are of major importance in the pathophysiology of restenosis. Macrophage recruitment is associated in other clinically important disorders such as myocardial infraction (MI) and endometriosis. We validated the hypothesis that systemic and transient depletion of circulating monocytes inhibits the inflammatory cascade. Monocytes/macrophage depletion was achieved with a systemic injection of nanoparticulated dosage forms (PLGA-based NP and liposomes) containing bisphosphonates (BP), which were formulated for effective phagocytosis. Following phagocytosis the vesicles discharge their encapsulated drug like a Trojan Horses, inactivating the cell with no effect on non-phagocytic cells. We investigated the effect of different BP, NP type (polymeric or liposomal), and size on the formulation properties, biodistribution, and monocytes sub-populations. Bioactivity and mechanism was examined in tissue cultures, and in animal models of restenosis, MI and endometriosis. Partial and transient depletion of blood monocytes following NP systemic injection correlated with the therapeutic effect. Phase I clinical studies confirmed the safety and potential efficacy of the nanoparticulate delivery system leading to ongoing Phase II clinical studies in stented patients.

RNAI NANOMEDICINES: CHALLENGES AND OPPORTUNITIES WITHIN THE IMMUNE SYSTEM.

Dan Peer[1,2]

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RNA interference (RNAi)-based approaches have greatly contributed to better understanding of gene expression and function *in vitro*. The capability to apply these strategies *in viv*o in order to validate the role of specific genes in normal or pathological conditions, and to induce therapeutic gene silencing, opened new avenues for utilizing RNAi as a novel therapeutic modality. However, the translation of RNAi from an effective genomic tool into clinical practice has been hindered by the difficulty to deliver RNAi molecules into their target tissues by systemic administration, especially to hematopoietic cells. In this presentation, we will describe some of the challenges and opportunities in modulating leukocytes using RNAi. Major focus will be given to the integrin-targeted stabilized nanoparticles (I-tsNP) strategy, which utilizes leukocytes' integrins for the delivery of siRNAs exclusively to cells of the immune system. Examples from inflammatory bowel diseases, viral infections and blood cancers will be discussed.

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NOVEL FIBRILLAR INSULIN FORMULATIONS FOR ORAL ADMINISTRATION: FORMULATION AND IN-VIVO STUDIES IN DIABETIC MICE

Yaron Dekel[1], Yifat Gliksam[2], Inbar Elron[2], Rimona Margalit[2]

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Oral delivery of protein and peptides is the "holly grail" of drug delivery. The oral route is the preferable approach for drug administration but the very low bioavailability of proteins (Insulin ~0.5%) is making this path still unreachable. In our attempts to overcome the inherit problems of oral protein delivery, we developed and evaluated in diabetic ICR mice two novel insulin particle formulations, possessing different structures yet sharing some common features. The main similar feature is the utilization of fibrillar insulin, i.e ordered reversible insulin aggregates, instead of using the naive peptide. The loading capacity of both formulations reached up to completion over the insulin range of 1–10 mg/ml, indicative of the predominantly hydrophobic interaction between the forming fibrils and the particles. Both formulations demonstrated high retention of insulin loads in harsh proteolytic and simulated gastro-intestinal environments. The formulations were tested *in-vivo* on diabetic ICR mice (Diabetes induced by Streptozotocin). After a single oral dose of each of the formulations, a follow up of the mice blood glucose level was conducted over the time span of 8 h in two different protocols: the conventional protocol (12 h pre-fasting and 8 h fasting); our revised protocol (no pre-fasting, meal at t=4 h). In both cases, initial blood glucose levels (BGL) were 400–600 mg/dL and the novel formulations generated a continuous reduction of BGL. Results in the revised protocol, that mimics better human eating habits, were more pronounced, providing stable (over several hours) glucose reductions approaching non-diabetic BGL values. The results obtained with both formulations merits further research into the molecular mechanism of action *in vivo*. Future perspectives on fibrillar protein formulations for other routes of administration and pathologies will be discussed as well.

TRANSFERRIN RECEPTOR AS A POTENTIAL TARGET MOLECULE FOR THE LOCAL TREATMENT OF INFLAMMATORY BOWEL DISEASES VIA THE LUMINAL ROUTE

Efrat Harel [1], Abraham Rubinstein [1], Yechezkel Barenholz[2], Aviram Nissan[3] and Boaz Tirosh [1]

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Purpose: To evaluate the role of transferrin receptor (TfR) as a novel target for the local, luminal drug treatment of IBD.

Methods: TfR expression in the colonic mucosa of either IBD patients or DNBS-induced rat model was evaluated by immunofluorescent staining. To assess the effect of induced inflammation on TfR levels at the surface of colon epithelial cells, Caco-2 cell line were analyzed by flow cytometry after their incubation with a variety of proinflammatory cytokines. Fluorescently-tagged immunoliposomes were prepared by conjugating negatively charged liposomes with anti-TfR antibody. Their specific binding and cellular uptake was measured in Caco-2 cell, in the presence or absence of proinflammatory cytokines, by fluorescence microscopy.

Results: The colonic mucosa of both IBD patients and DNBS induced rats expressed elevated levels of TfR. The major cytokine promoting TfR expression on the surface of the Caco-2 cells was TNFα. Using anti-TfR fluorescently-tagged immunoliposomes, it was found that the immunoliposomes accumulated, better then naked liposomes, in the Caco-2 cells in a concentration dependent manner.

Conclusion: Cell surface TfR is upregulated in IBD. This apical expression occurs, most likely, due to a direct involvement of proinflammatory cytokines. This trait could be exploited for targeting drugs to the inflamed gut mucosa via the luminal route.

Acknowledgment:
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PATENT PROTECTION FOR INNOVATIONS IN CONTROLLED RELEASE DRUG TECHNOLOGY.

Joseph I. Wyse and Keren Hagai,

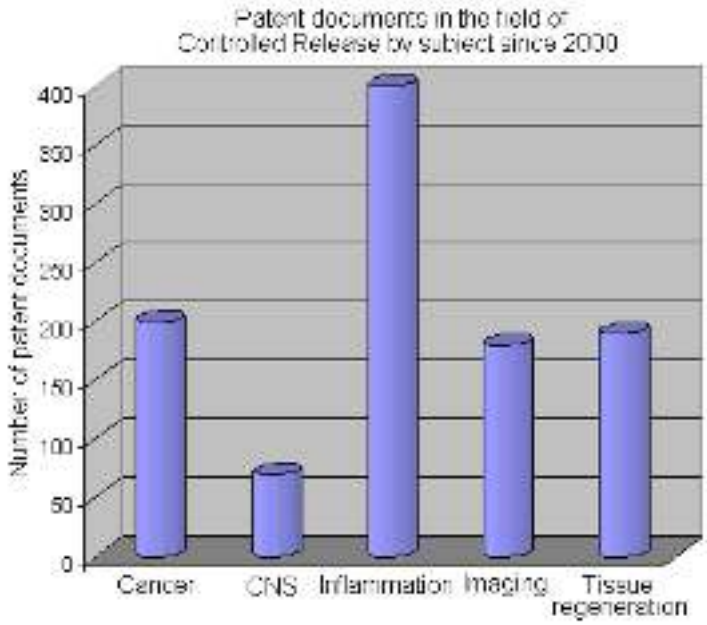
Eyal Bressler & Co. Patent Attorneys

Intellectual Property (IP) Patent protection is crucial for the commercial success of any development in Pharma, so that the innovators can exploit their monopoly on patented inventions to offset R&D costs and make profits.It is equally important for Pharma companies and research groups to be aware of competitor patent activity before investing large amounts of money on efforts which, whilst scientifically sound, may not have freedom to operate from a patent perspective.

Many old drugs are shedding their patent protection in the short to medium term. It has long been recognised that these "off patent" drugs can have a new lease of clinical and commercial life imbued in them by maximising their "Second Use" and Controlled Release potential.

At the same time, many new drugs are being designed with Controlled Release as a drug delivery strategy, early in the product lifecycle. There is also an increasing tendency to regard the active molecule and the vehicle delivering it, as a unified entity, as well as other strategies focused on delivery systems *per se*.

There is rich and varied patent activity in the field of Controlled Release, especially in the growth areas of Cancer, CNS, Inflammation, Imaging and Tissue Regeneration. Data is presented showing this activity, and important trends are identified. The patent portfolios of leading players in Controlled Release are surveyed. Strategies are suggested for maximising the patent protection of advances in the field, and for identifying the maturity of a technology from a patent perspective.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.



ELIMINATING NEEDLES: TRANSDERMAL DELIVERY OF PEPTIDES AND OLIGONUCLEOTIDES THROUGH SKIN

Galit Levin

VP R&D Pharma, TransPharma-Medical

TransPharma's ViaDerm drug delivery system provides a cost-effective, easy-to-use, self-administered solution that enables the safe, reproducible and accurate delivery of a broad range of active material candidates, including peptides, proteins and oligonucleotides. The system incorporates a handheld electronic device, which creates microscopic passageways through the outer layer of the skin allowing for intradermal and/or transdermal delivery of a wide variety of drugs from a patch or other topical dosage forms. One example of a successful application of the system is ViaDerm-hPTH (1-34), a novel transdermal hPTH (1-34) product which is designed to help people manage their osteoporosis by eliminating the need for daily painful injections. The product has successfully completed a phase 2A clinical study and is jointly developed by TransPharma and Eli Lilly. Other applications, such as extended delivery of GLP-1 agonist as well as the delivery of genetic material into skin cells will be presented too.

DESIGNING LONG ACTING RECOMBINANT PROTEINS: FROM BENCH TO CLINICS

Fuad Fares

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Peptides are used clinically in the treatment of many diseases. One major issue regarding the clinical use of many peptides is their short half-life span in the body, due to the rapid clearance from the circulation. The low stability of peptides has thus often posed a difficulty to researchers and hindered their adoption in potential medical applications. Thus, at the clinical level, there is a need for a regime of frequent injections of the peptides into the patients to overcome this low stability factor. The major strategies for overcoming this problem by pharmaceutical companies are based on chemical techniques and using specific peptidase inhibitors or cocktails. To overcome this problem, we used genetic engineering techniques that have been found successful for designing long acting hormones. Using site-directed mutagenesis and overlapping PCR techniques, we succeeded to add the signal sequence of *O*-linked oligosaccharides to the coding sequence of the hormones. The cassette gene that has been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin β (hCGβ) subunit. The CTP contains 28 amino acids with several proline and serine residues and four *O*-linked oligosaccharide recognition sites. It was postulated that the *O*-linked oligosaccharides add flexibility, hydrophilicity and stability to the protein. On the other hand it was suggested that the four *O*-linked oligosaccharides play an important role in preventing plasma clearance and thus increasing the half-life of the protein in circulation. Using this strategy we succeeded to ligate the CTP to the coding sequence of follitropin (FSH), thyrotropin (TSH), erythropoietin (EPO) growth hormone (GH), interferon beta and thus to increase the longevity and bioactivity of these proteins *in-vivo*. Interestingly, the new analog of FSH was found not immunogenic in humans and it is already passed successfully clinical trials phase III and approved by The European Commission (EC). In addition, our results indicated that long acting GH is not toxic in monkeys and it passed successfully clinical trials phase I and the protocol for phase II was approved. The preliminary results regarding interferon beta seem to be promising. Thus, using this technology seems to be promising in designing long acting peptides. Development of long acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in clinical protocols.

CONTROLLED RELEASE IN WATER PURIFICATION SYSTEMS

Avi Domb

Hebrew university of Jerusalem

LOCAL STIMULATIONS IN 3D FOR CONTROLLED DIFFERENTIATION AND ORGANIZATION OF EMBRYONIC STEM CELLS

Shulamit Levenberg

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The rapidly increasing demand for organ and tissue transplantation has promoted tissue engineering and stem cell research as promising approaches. Tissue engineering combines cells, growth factors and 3D scaffolds for repair and regeneration of biological tissues. To advance tissue engineering research, scaffold properties must be optimized for a given application and cell type. This includes chemical and mechanical properties, shape, and structure and degradation rate. In addition co-culture approaches are required to allow organization of complex tissue structures. Endothelial cell co-cultures are important for inducing vascularization of engineered tissues. Our experiments in engineered skeletal and cardiac muscle tissue indicate that endothelial cells promoted differentiation and organization of the co-cultured myoblasts. Endothelial 3D tubular networks were formed within the tissue and shown to promote vascularization upon implantation. Our recent results using pancreatic islets co-cultures further support the inductive effect of endothelial vessels on islets survival in vitro and in vivo. Given the attractive potential of human embryonic stem cells in tissue regeneration we evaluate the ability to differentiate the cells and induce their 3D organization toward formation of complex tissues. Biodegradable, growth factor-eluting nano-fibers are used to study embryonic stem cells process in 3D models. Differentiation of the cells is further studied in micro systems to allow the precise localization of a growth factor. These techniques can provide a tool to investigate cell-cell signaling between adjacent embryonic stem cells by maintaining a constant gradient of growth factors in the surrounding culture medium.

Understanding embryonic stem cells differentiation and 3D cellular communications can lead to advances in cell therapy and tissue engineering and facilitate organ and tissue regeneration.

CELLS, GELS, TISSUE, AND ISSUES: NOVEL HYDROGELS AS SCAFFOLDS DESIGNED WITH AFFINITY-BASED DRUG DELIVERY

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In this work we present the development of hydrogels for drug delivery applications, based on the affinity of drugs to poly(ethylene glycol) (PEG) conjugated albumin. Both of these constituents are widely available, inexpensive and FDA approved. This hydrogel system is photo-polymerizable, thus allowing minimally invasive administration for local sustained drug release at the target tissue without covalent modifications of the drugs. We used PEG and albumin to produce a few variations of hydrogels, including wild-type albumin mixed with PEG, multi-PEGylated albumin, and mono-PEGylated albumin. Furthermore, we performed PEGylation reactions using PEG of different molecular weights, and integrated the PEGylated albumin precursor with PEGylated fibrinogen, which is known for its tissue regeneration and cell adhesion properties. Drug release experiments were designed and performed in order to study the effect of several hydrogel design parameters on the release kinetics from the hydrogels, including: the drug affinity to albumin; the molecular weight of the drug; the molecular weight of the PEG conjugated to albumin; the addition of free PEG-DA molecules to the hydrogel matrix; the molecular interactions between the polymer and the protein, i.e. degree of PEGylation; and the presence of a degrading enzyme. The degradation properties of the hydrogels, as well as their biocompatibility, were also examined *in vitro* and *in vivo*. Results of these experiments indicated that mono-PEGylation of albumin conserves the drug binding properties of the protein while allowing its covalent conjugation within a hydrogel matrix. PEGylation reaction using lower molecular weight PEG resulted in slower drug release kinetics. Thus, the ability to control the release properties of PEGylated albumin hydrogels was obtained through the PEGylation degree, the molecular weight of PEG conjugated to the protein, and the addition of free difunctionalized PEG molecules. Moreover, by using a composite hydrogel system including both mono-PEGylated albumin and PEGylated fibrinogen, we showed sustained drug delivery properties from a hydrogel that also supports cell culture and tissue regeneration. This combination opens up a broader spectrum of potential uses, in cases where drug release and tissue regeneration are required. The rate of both processes may be optimized through the composition of the hydrogel to give an accurate treatment for such specific needs.

SEQUENTIAL, MULTI-COMPONENT-RELEASING SCAFFOLDS FOR TISSUE ENGINEERING: FABRICATION BY THE NOVEL SOLVENT/NON-SOLVENT SINTERING TECHNOLOGY (*)

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Most of the currently available biodegradable scaffolds required for tissue engineering procedures provide only physicochemical support to the proliferating cells. Yet, also required is the appropriately timed and localized supply of numerous growth factors, acting as the source of biochemical information. This issue was considered in some more recent developments, offering the option for essentially concomitant release of two growth factors. Here we report on a novel technology by which porous, biodegradable scaffolds that perform all the above mentioned functions can be conveniently produced. This technology relies on the use of a scientifically and technologically sound solvent/non-solvent sintering procedure. Moreover, it is performed under mild conditions that preserve the integrity and activity of incorporated agents and it also allows for conveniently fine-tuning the various release schedules, as desired. As a representative example of implementation of this technology, nanoparticles of poly (suberic anhydride) containing all-*trans* retinoic acid (atRA) and microparticles of poly (D,L-lactic acid-*co*-glycolic acid) containing bovine serum albumin (BSA, as a model compound) were co-sintered, using methylene chloride / diethyl ether as the solvent and diethyl ether as the non-solvent. The resulting scaffold is characterized by appropriately high porosity (about 50 %) with interconnected pores of the size 50-500 μm, and by a compressive modulus of 200 kPa. This construct was shown to release 100% of the contained atRA within 7 days and 10% of the contained BSA after one month, thus essentially achieving the required sequential mode of operation. Also, when scaffolds of this type were seeded with fibroblast cells, the latter adhered to the surface of the sintered particles and maintained their spherical shape. Thus, the developed approaches and their implementation technology offer a most convenient and versatile strategy for producing "intelligent" platforms intended to carry and deliver biologically active materials. Moreover, this approach can be further extended to developing advanced modalities for scheduled release and delivery of other multi-component bioactive payloads, such as drugs.

Keywords: Sequential release; Scaffolds; Growth factors; Tissue engineering; Solvent sintering

(*)*Taken in part from the M.Sc. Thesis of Nitsa Ne'eman, submitted to the Technion, Haifa, Israel*

NOVEL ANTIBIOTIC-ELUTING COMPOSITE STRUCTURES FOR WOUND HEALING APPLICATIONS: IN-VITRO AND IN-VIVO STUDY

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About 70% of all people with severe burns die from related infections, despite advances in treatment regimens and the best efforts of nurses and doctors. Although silver-eluting wound dressings are available for addressing this problem, there is growing evidence of the deleterious effects of such dressings in delaying the healing process due to cellular toxicity. Local administration of antibiotics at the wound can give relatively high but local doses of antibiotics, avoiding any toxicity issues that arise when the same amount of antibiotic passes through the body.

Our new composite structures, based on a dense polymeric mesh and a porous Poly(dl-lactic-co-glycolic acid) matrix, is designed to protect the wound until it is no longer needed, after which it dissolves away by chemical degradation to non-toxic products. Avoiding constant wound cleaning and redressing should enable the body to better cope with healing and reduce patient pain and suffering. The porous matrix preparation is based on freeze drying of inverted emulsion's technique. Structuring of the porous matrix enables controlling the relevant physical properties of this platform for wound healing applications, i.e. drug release profile and water vapor transmission rate.

The antibiotic release profiles from the composite structures generally exhibited an initial burst effect accompanied by a decrease in release rates with time over periods ranging from several days to 30 days, depending on the emulsion's formulation. Higher Organic:Aqueous phase ratios, polymer content and molecular weights, all reduced the burst release of the antibiotics from the wound dressings and prolonged their release due to changes in the porous matrix structure. The effect of antibiotic release on bacterial inhibition was studied and cell cytotoxicity was examined. The antibiotic release resulted in a 99.99% decrease in the viable counts of P. aeruginosa and S. albus at very high initial inoculations of 10^7-10^8 CFU/mL after only one day, while such a decrease in S. aureus was obtained within 3 days. Bacterial inhibition zones around the dressing material were found to persist for 2 weeks, indicating a long-lasting antimicrobial effect. Despite severe toxicity to bacteria, the dressing material was found to have no toxic effect on cultured fibroblasts, indicating that our new antibiotic-eluting wound dressings represent an effective option for selective treatment of bacterial infections. In-vivo tests of the new wound dressings in a guinea-pig contaminated deep second degree burn model demonstrated 40% faster healing and better esthetical results (less wound contraction) compared to a conventional dressing material. Thus the newly developed wound dressing offers a potentially valuable and economic approach to treating the life-threatening complication of infections related to burns.

KEYWORDS: fibroblast, gentamicin, poly-(DL-lactic-co-glycolic acid), infection, wound healing.

SEQUENTIAL DELIVERY OF IGF-1 AND HGF FROM INJECTABLE ALGINATE BIOMATERIAL PROMOTES MYOCARDIAL REPAIR AFTER MYOCARDIAL INFARCTION

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Background and aims: Various factors are known to improve tissue salvage and elicit a favorable course of tissue repair after myocardial infarction (MI)**.** However**,** the proper spatio-temporal delivery of these factors to infarcted hearts represents a challenge**.** We hypothesized that dual delivery of insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) by injectable affinity-binding alginate biomaterial would maximize their therapeutic effects, leading to a more favorable course of tissue restoration after acute MI.

Methods and results: MALDI-TOF analysis showed that bioconjugation of IGF-1 or HGF with alginate-sulfate protects the proteins from enzymatic proteolysis. The release profile of both factors from the affinity-binding alginate hydrogel revealed a sequential release pattern (IGF-1 followed by HGF)**,** for a period of 1 week. The factor bioactivity was confirmed by Western blot analysis for induced phosphorylation of AKT and ERK1/2 for IGF-1 and HGF, respectively, and prevention of cardiac cell death in oxidative stress model in isolated rat neonatal cardiomyocyte cultures. Short (1 week) and long-(4 and 8 weeks) term effects of dual (IGF-1/HGF) delivery by injectable affinity-binding alginate were tested in a rat model of acute MI. After 4 weeks, the treatment with sequentially-delivered proteins increased scar thickness, prevented infarct expansion and reduced fibrosis. This treatment also increased mature (α-SMA-positive) blood vessel density and area. Active caspase-3 staining showed that dual delivery of IGF-1/HGF also reduced apoptosis at the infarct. These effects were preserved for 8 weeks. The sequential delivery of IGF-1/HGF induced the greatest number of Ki67-positive myocytes, 1 week after treatment, and also resulted in higher incidence of GATA-4 positive cell clusters at 4 weeks. 2D echocardiography showed that IGF-1/HGF in affinity-binding alginate prevented left ventricular (LV) dilatation, as shown by attenuated increase in LV end systolic and diastolic diameters, when compared to the treatment with soluble factors, 4 weeks after MI/injection.

Conclusions: The dual delivery of IGF-1/HGF from affinity-binding alginate biomaterial represents an applicable strategy to treat MI. The affinity-binding mechanism preserved factor bioactivity and enabled its protection in the harsh MI environment. This treatment also showed a marked long-term therapeutic efficacy at various levels, including prevention of adverse LV remodeling, induced angiogenesis and improved cell survival. Finally, the developed approach showed a potential to induce endogenous regeneration of cardiac muscle.

Acknowledgement: The research was supported by grants from the Israel Science Foundation (793/04 to SC and 1368/08) and European Union FWP7 (INELPY). We thank Radka Holbova for excellent technical assistance. Prof. Cohen holds the Claire and Harold Oshry Professor Chair in Biotechnology.

Keywords: affinity binding, alginate hydrogel, hepatocyte growth factor, insulin-like growth factor-1, myocardial regeneration

The results presented in the abstract are part of a PhD thesis.

POSTER SESSION

P-1

NOVEL MUCOADHESIVE SYSTEM BASED ON SULFHYDRYL-ACRYLATE INTERACTIONS

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The concept of mucoadhesion was first introduced in the early 1980's as a new approach to improve drug release, targeting and absorption. Mucoadhesion is defined as the ability to adhere to the mucus gel layer. The first and most common line of mucoadhesive polymers were based on a polymer-mucin interaction through physical and/or chemical non-covalent bonds such as hydrogen bonds, Van-Der Waal forces, ionic interactions and/or chain entanglement. However, attempts have been made to improve the mucoadhesive properties *via* covalent linking such as disulfide bonds. Although dry carriers prepared from thiomers display high adhesion, our previous study have shown that mucoadhesive system based on alginate-thiol molecules were not able to display their advantages in hydrated environment.
In order to overcome this limitation, a novel mucoadhesion approach is proposed. It is based on the ability of molecules carrying electronegative vinyl end group to covalently associate with electronegative neighboring groups, in a reaction termed Michael type addition, which can take place in physiological environment. The underlying hypothesis was that the ability of acrylate chemical end groups to covalently associate with sulfide end groups under mild conditions could be used to chemically attach molecules carrying such groups to the mucus. This hypothesis was first verified by the formation of covalent bonds between polyethylene glycol di-acrylate (PEG-DA) and mucin using nuclear magnetic resonance (NMR) and rheology experiments. In addition, the adhesion to fresh tissue and drug release ability were examined.
This approach was further developed by synthesizing a novel biomaterial consist PEG-acrylate attached to an alginate backbone. Such a polymer combines the strength, simplicity, and gelation ability of alginate with the mucoadhesion properties arising from the PEG's characteristics as well as the acrylate functionality. We have synthesized, verified the formation of the desired product by NMR and evaluated its ability to function as a novel mucoadhesive material for control drug release by means of tensile and *in vitro* release measurements.

Keywords: mucoadhesion, drug release, alginate, PEG, acrylate.

This abstract describes the work done by the PhD student Maya Davidovich-Pinhas.

P-2

NANOPARTICLES EMBEDDED IN ALGINATE HYDROGELS: AN INNOVATIVE APPROACH FOR SUSTAINED RELEASE OF HYDROPHOBIC DRUGS

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Over 40% of the drugs developed in the past two decades are extremely hydrophobic. Lipophilic drugs can act as efficient therapeutic agents, since they can penetrate the hydrophobic cell membrane as opposed to hydrophilic drugs. However, their low bioavailability restrains their implementation. We present an innovative methodology for sustained delivery of hydrophobic drugs using composite hydrogels, prepared by embedding oil-in-water microemulsions in hydrophilic hydrogels. The hydrophobic nature of the microemulsion core enhances the solubilization of hydrophobic drugs, while the crosslinked matrix could be readily used as a solid controlled delivery vehicle.

A microemulsion (ME) was formulated from pharmaceutical accepted components: Tween-80 and Span 20 as the surfactant mixture, and isopropyl myristate as the oil component. The droplets diameter was shown to be about 10 nm by dynamic light scattering and small angle X-ray scattering (SAXS). Combining the ME with alginate solution and crosslinking with calcium ions resulted in a clear hydrogel. The model drug, Ketoprofen, precipitated from the alginate hydrogel, but the drug-containing composite hydrogel was clear and macroscopically homogenous (figure 1), suggesting that the model drug is more soluble in the ME-composite gel than in the gel alone. The nanostructure was investigated by SAXS; scattering plots indicate that oil droplets indeed exist in the composite hydrogel, which may well explain the increased soubility of the drug in the hydrogel. Release profiles from composite gels exhibit gel-composition dependency; these profiles demonstrate the applicability of this system as a controlled delivery vehicle. The methodology of incorporating microemulsion droplets into alginate hydrogels for the purpose of increasing drug solubility could be applied to more drugs. Moreover, other hydrophilic polymers can be utilized to create composite hydrogels. Other systems have to be examined carefully, as the polymer and the drug could affect the stability of the microemulsion. Yet, we believe that composite hydrogels hold great potential for enhancing the solubility of hydrophobic drugs.

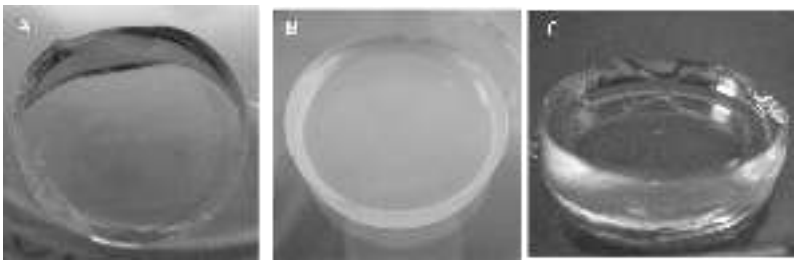

Fig. 1: Alginate hydrogels, with25 mg/ml alginate and 15 mM Ca-EGTA. (A) No drug, (B) KT 1 mg/ml, (C) Composite gel with 1 mg/ml KT.

Keywords: Hydrophobic Drugs; Hydrogels; Alginate; Microemulsions

P-3

REDUCTION OF CHEMICAL BONDS BY BACTERIA OF THE COLON

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Specific delivery of oral drugs to the colon is important in addressing several types of medical conditions of the colon such as cancer and Crohn's disease, as well as for systemic applications. In particular, biodegradable delivery systems (either prodrugs, coatings or matrices) are considered to be potentially the most accurate. Colonic bacteria are able to perform a large number of chemical reactions unique to this part of the GI tract, the most explored of which are hydrolysis and reduction. Although quite a few delivery systems were designed based on the familiar reduction of azo bonds, other reducible groups with potential use in colonic drug delivery were left almost entirely unexplored. Further more, in many of the azo delivery systems, little thought was given to the structure of the azo molecule, leading to slow reduction rates.

In the present work, we compared the rate of reduction of three chemical bonds with the potential of being used in colonic delivery systems: azo, disulfide and nitro, in rat cecal contents. The molecules used were similar in structure to the active part of the commercial prodrug Sulfasalazine. Sulfasalazine is a known clinically used azo prodrug with efficient reduction in the colon and was used as a reference compound in our experiments. We further explored the reduction of different nitro compounds to study the structure activity relationship (SAR) of this reaction. In contrast to many other publications on reduction by the bacteria of the colon, our work was based on following the accumulation of the final reduction product, and not the disappearance of the starting compound.

The results show, that disulfide and nitro reduction rates are comparable to that of azo having similar structures: k/k_0(Sulfasalazine)=0.3-2.4 for all three chemical groups. Thus these bonds could potentially be used in drug delivery systems being reduced with a rate comparable to that of Sulfasalazine.

We conducted a SAR study using five different nitro compounds. Using the Hammet equation, a surprisingly high correlation was observed for the reduction of nitro compounds, with electron-withdrawing groups accelerating the process: ρ=0.5285, r^2>0.99. Two of the nitro compounds - containing *p*-Cl and *m*-CF_3 groups, were found to undergo reduction faster than Sulfasalazine. The results emphasize the potential of nitro groups in colon specific drug delivery.

Key words: Colon-specific drug delivery, reduction, azo, nitro, disulfide.

* The abstract describes a student's research results.

P-4

COMPARISON BETWEEN THE PHARMACOKINETIC PROPERTIES OF TWO NON METABOLIC LONG CHAIN FATTY ACID ANALOGS FOR THE THERAPY OF METABOLIC SYNDROME

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Purpose: Free fatty acids (FFA) are a major energy source in human nutrition as well as potent regulators of physiological metabolic signaling. Various non metabolic analogs of FFA have been developed as potent therapeutics mimicking the features of native FFA with increased pharmacodynamic efficacy and altered pharmacokinetic properties. Mαα and Mββ, two non metabolic analogs that differ in the location of their methyl group, exhibited marked hypolipidemic and insulin sensitizing effects in preclinical studies. Considering the high therapeutic potential as a treatment for the Metabolic Syndrome, the aim of this work was to compare these compounds in terms of pharmacokinetic parameters including metabolic pathways, tissue distribution and oral bioavailability.

Methods: Pharmacokinetics and oral bioavailability of Mαα and Mββ was assessed in male Wistar rats following single IV and PO administration. LC-MS analysis was used for compounds quantification. Intestinal permeability properties of Mαα and Mββ were measured in Caco-2 cells and permeation flux ratio was evaluated. In-vitro enzymatic metabolism was assessed using rat pooled liver microsomes. Tissue distribution was examined using radiolabeled compounds. Pharmacokinetic parameters were determined using compartmental analysis.

Results: A two-compartment model with an elimination half-life of approximately 3 hours describes the pharmacokinetics of both analogs following intravenous administration. Plasma concentrations following oral doses are higher for Mαα in comparison to Mββ with peak concentrations of 77±40 and 39±5 g/ml respectively, and similar absolute oral bioavailability of 55%. Both compounds exhibit high permeability through CaCo2 monolayer with influx ratios of 5 and 1 for Mαα and Mββ respectively. Systemic clearance and volume of distribution at steady state of Mββ were higher than Mαα (39±2 and 20±3 ml/kg/hour, 140±20, 57±6 ml/kg respectively). Mββ was more extensively metabolized by rat liver microsomes and was distributed to liver, fat and muscle tissues while Mαα was found mainly in the liver.

Conclusion: Mαα lower clearance and diminished susceptibility to oxidative enzymatic metabolism as well as protein mediated intestinal influx are ascribed to methyl substitution on α to carboxyl position. These relatively minor structural changes also alter tissue distribution and play a role in fatty acid analogs pharmacokinetics.

*The abstract describes this student's research results.

P-5

SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEMS (SNEDDS) FOR IMPROVED BIOAVAILABILITY OF BCS CLASS 2 COMPOUNDS: EFFECTS ON SOLUBILIZATION, INTRA-ENTEROCYTE METABOLISM, AND P-GP EFFLUX

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Purpose: Numerous BCS Class 2 compounds exhibit low and erratic bioavailability. SNEDDS was previously reported to improve bioavailability of Class 2 compounds. SNEDDS previously developed by our group significantly improved oral bioavailability of Cyclosporine A in humans. Our study aims to elucidate the mechanisms involved in the improved bioavailability achieved by incorporation of these compounds into SNEDDS.
Methods: SNEDDS of amiodarone (AM) and tacrolimus (TCR), Class 2 compounds, were developed and optimized. PK was assessed *in-vivo*. Permeability was evaluated *in-vitro* (Caco-2) and *ex-vivo* (Ussing chamber). Solubilization was assessed by *in-vitro* dynamic lipolysis model. LDH assay was used for cytotoxicity evaluation. The effect on intraenterocyte metabolism was evaluated in CYP3A4 microsomes. P-gp efflux inhibition was determined *in-vitro*, using talinolol - a P-gp substrate that is not subjected to intraenterocyte metabolism. Transepithelial electrical resistance and mannitol permeability were measured to assess tissue damage.
Results*: In-vivo*, AM-SNEDDS showed significantly higher AUC vs. AM (9.23±0.83 vs. 6.28±3.0 hr*µg/ml) with significantly higher C_{max} following PO administration. No effect on bioavailability was found when AM was administered 2h subsequent to blank SNEDDS. AM-SNEDDS exhibited more consistent absorption. Similar findings were observed in TCR studies, coupled with prominent reduction in plasma concentrations variation coefficient. Talinolol Caco-2 studies resulted in significantly higher permeability coefficient (Papp) of talinolol-SNEDDS vs. talinolol. AM-SNEDDS *ex-vivo* Papp was significantly higher than AM. Higher solubilized AM concentrations were found following AM-SNEDDS lipolysis vs. AM (90.5±1.33% and 59.1±9.45 % of initial conc. respectively). Significantly higher intact AM concentrations remained following incubation of AM-SNEDDS vs. AM with CYP3A4 (102.4±5.61% and 68.57±1.17%respectively). Adding blank SNEDDS to testosterone before incubation also resulted in significantly reduced testosterone metabolism. SNEDDS membrane toxicity was negligible and no damage to tissue integrity or tight junctions structure was observed.
Conclusions: Our SNEDDS not only improves solubilization, but also reduces intra-enterocyte metabolism and P-gp activity. Thus, our SNEDDS increases bioavailability of Class 2 compounds and reduces their typical high variability in bioavailability by multi-processes mechanism. Nonetheless, the effect of our SNEDDS on bioavailability is reversible, and can't be attributed to interruption of the GI wall structure or cell membrane damage. These notable findings contribute to our understanding of the investigated phenomenon of the *in-vivo* impact of SNEDDS on oral bioavailability. Better mechanistic understanding of these fundamental processes will further lead to finding intelligent pharmaceutical solutions for the enhanced bioavailability and reduced variability of Class 2 drugs and drug candidates

Keywords: bioavailability, SNEDDS, P-gp efflux, intra-enterocyte metabolism, BCS Class 2

*This research was conducted in the framework of Anna Elgart's PhD dissertation.

P-6

NOVEL ORALLY AVAILABLE ANTI-HIV DRUG LEAD CANDIDATE

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Purpose: To develop a novel, low molecular weight, orally available drug lead that mimics the CD4 recognition site and blocks the binding of the viral gp120.

Methods: Based on cycloscan approach, the non-continuous active region of CD4 was converted to a backbone cyclic (BC) peptide that mimics the active region of the target protein. Additional chemical modifications were performed to synthesize a small macrocyclic CD-4 mimetic lead. The macrocyclic molecule named CG-1 was evaluated for its intestinal permeability using the Caco-2 model and ex-vivo in rat intestinal model. Its stability was evaluated using intestinal brush border membrane vesicles (BBMV's) and liver microsomes. The anti-HIV activity was evaluated by multinuclear activation of a galactosidase indicator (MAGI) assay. Its pharmacokinetic profile following intravenous and oral administration was also investigated.

Results: While the permeability coefficient (Papp) through Caco-2 monolayer was low, the Papp was significantly higher in the ex-vivo model ($1.2\pm0.09x10^{-6}$, $1.8\pm0.15x10^{-5}$ cm/sec±SEM, respectively). We further studied the permeability mechanism of CG-1 in the ex-vivo model. The Papp of apical-to-basolateral transfer (Papp A-B) was an order of magnitude higher than Papp B-A. This directionality indicates a possible transporter-mediated mechanism. CG-1 was stable in BBMV's (>85% remained at the endpoint), and partially metabolized by human and rat liver microsomes (<60% remained at the endpoint). Addition of ketoconazole (CYP3A4 inhibitor) significantly improved its stability. The in-vivo studies showed a good oral bioavailability and half life of 73 minutes. CG-1 inhibited HIV infection in Hela cells at 8μM.

Conclusions: The cycloscan approach resulted in a small macrocyclic drug lead (MW <500 g/mole) with improved pharmacodynamic and pharmacokinetic properties. The macrocyclic molecule named CG1 has a good oral bioavailability and stability to enzymatic degradation with anti-HIV activity in the low μM range.

Keywords: HIV, peptidomimetic, metabolic stability, intestinal permeability, bioavailability

P-7

INVESTIGATION OF THE ORAL AVAILABILITY AND IN VIVO ANALGESIC ACTIVITY OF NOVEL BACKBONE CYCLIC TETRAPEPTIDES

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According to recent clinical studies, one in five Europeans suffers from chronic pain despite the availability of various analgesic treatments. Thus, the need for new analgesic drugs is immense. Several classes of compounds derived from endogenous opioid ligands have been discovered. Among these is dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), a heptapeptide derived from amphibian skin, which has high affinity and selectivity towards μ-opioid receptors and antinociceptive activity. The N-terminal tetrapeptide of dermorphin is the minimum sequence required for opioid activity, but the short fragment is less potent than the native heptapeptide. However, the substitution of the D-Ala2 residue with D-Arg and the Gly4 residue with sarcosine (Sar), markedly enhances the potency of the tetrapeptide., named TAPS, which induced a stronger and more prolonged effect than morphine when administered intracerebroventricularly or subcutaneously (SC).

In order to enable oral administration, peptides must be stabilized towards enzymatic degradation and have intestinal permeability.

We describe here a backbone cyclic (BC) library of five tetrapeptides derived from TAPS, named Taps #2-6, differing in the length of the bridge linkers. The pharmacological activity of this library was analyzed for binding affinity towards δ- and μ- opioid receptors. Selected peptides were further investigated for *in vivo* activity in mice using the thermal tail flick and tail withdrawal models. The peptides were investigated *in vitro* for permeability in Caco-2 model and for enzymatic stability in the gastrointestinal tract using rat pooled brush-border membrane vesicle (BBMV) enzymes.

The opioid receptor affinity varied among the peptides. While Taps #3 and #5 showed low affinity towards μ- receptor in μM concentrations, the affinity of Taps #2, #4 and #6 towards the same receptor was high. Only Taps #4 had some affinity towards the δ receptor. All five BC peptides exhibited stability towards enzymatic degradation in BBMVs when compared to the linear TAPS parent peptide. Taps #6 exhibited improved permeability in the Caco2 cell model, while the permeability coefficient values of the other analogs, along with the linear parent peptide, were similar to mannitol, a marker of passive paracellular transport.

Taps #2, #4 and #6 were further evaluated for their pharmacological activity *in vivo* when administered IP to mice. The analgesic activity induced by Taps #6 in the tail flick model was similar to that induced by methadone (10 mg/kg), while Taps #2 and #4 did not display an antinociceptive effect. In addition, Taps #6 was examined in the tail withdrawal test, showing an antinociceptive effect stronger than methadone and with a higher peak effect.

In this study we present an analgesic, enzymatically stable backbone cyclic tetrapeptide with promising potential as an orally administered treatment for pain.

Acknowledgement: this project is funded by ActiveP Ltd.

Keywords: pain, analgesia, backbone cyclization, peptide, oral bioavailability

*This abstract describes Avi Swed's research work.

P-8

DEVELOPMENT OF A NOVEL PROTEIN BASED ORAL DELIVERY SYSTEM FOR CONTROLLED RELEASE OF HUPERZINE A FOR A TREATMENT OF CNS RELATED DISEASES.

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Huperzine A (HupA) is a plant-derived alkaloid that has been used in China for centuries in the treatment of fewer, swelling and schizophrenia.

HupA is competitive, reversible and selective inhibitor of acetyl cholinesterase (AChE) as well as a NMDA receptor antagonist which protects the brain against glutamate induced damage. In addition, it has the ability to protect cells against hydrogen peroxide, beta-amyloid protein, ischemia and staurosporine-induced cytotoxicity. These pharmacological properties of HupA make it a useful drug candidate for the treatment of various central nervous system (CNS) related diseases.

HupA, is a compound that is chemically unique in comparison with other agents under study for Alzheimer's disease (AD). Its potency and duration of AChE inhibition rival those of approved drugs for treatment of AD, such tacrine, galanthamine, donepezil, and rivastigmine. Compared with other AChE inhibitors, HupA has better penetration through the blood-brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action.

There is already clinical data clarifying the therapeutic value of HupA in demention and AD, and preclinical data on its usefulness as a protective drug against epileptic convulsions and as prevention treatment against organophosphates (OP) intoxication. In addition there are some evidences for HupA antinociceptive activity.

The aim of the research is to develop an optimized drug delivery system for treatment of patients suffering from neurodegenerative diseases (AD and other memory disorders). This technology will enable a once daily treatment which is an essential component in the therapy of these age-related disorders that are increasing in the Western world.

Our findings support the rationale of a protein-based sustained release delivery system that will allow HupA absorbance all along the gastrointestinal tract, enabling a once daily treatment. A clinical bioequivalence study of HupA novel sustained release formulation in healthy volunteers is scheduled to start soon.

Keywords: Huperzine A, sustained release, delivery system, formulation

P-9

DRUG DELIVERY OF PROTEINS BY NANO-VESICLES MADE FROM BOLALIPIDS

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Background: Development of delivery systems targeted to the drugs' site of action is under extensive investigation with the goal of improving the pharmacokinetic and pharmacodynamic features of bioactive molecules (proteins, peptides, etc.). Bolaamphiphilic compounds, containing two selectively cleavable polar head groups attached to the two ends of a hydrophobic alkyl chain, form monolayer membrane vesicles that can be potentially used for targeted delivery of bioactive molecules, based on a high encapsulation capacity (vesicles with a thin membrane and a high inner volume), high stability and low tendency to fuse with other membranes (due to high energy barrier for lipid exchange) and possibility to trigger the release of the encapsulated material (via controlled destabilization of the bolaamphiphile head groups).

Aims: To investigate the ability of cationic vesicles made from novel bolaamphiphilic compounds to encapsulate proteins and to evaluate their potential for *in vivo* targeted drug delivery, with emphasis on delivery to the brain.

Methods: Vesicles encapsulating bovine serum albumin conjugated to the fluorescent marker fluorescein isothiocyanate (BSA-FITC) were prepared by film hydration and sonication from bolaamphiphilic compounds with acetylcholine head groups. Vesicles' size and morphology, encapsulation capacity, and *in vitro* stability were determined by TEM, DLS and fluorimetric measurements. Uptake of the vesicles by HeLa cells and the intracellular localization of the encapsulated protein were determined using FACS and confocal microscopy. *In vivo* tissue distribution of the encapsulated protein following systemic administration of the vesicles to mice was analyzed by histofluorescence analysis of tissue slices.

Results: Novel bolaamphiphilic vesicles had the average diameter of 50-100 nm, a positive ζ-potential (25-50 mV), and high encapsulation efficiency of BSA-FITC (up to 48%). The half-life of the vesicles was 3-5 hr, and their stability was significantly increased in presence of pyridostigmine, a quaternary choline esterase inhibitor, suggesting that hydrolysis of the head groups by serum choline esterase destabilizes the vesicles. Confocal imaging showed an efficient uptake of the vesicles and of the encapsulated BSA-FITC by HeLa cells at 37°C but not at 4°C. When the vesicles were injected intravenously to mice the encapsulated BSA-FITC was found in several tissues, including the brain. Brain uptake of the encapsulated BSA-FITC was increased when the mice were pretreated with pyridoistigmine, which inhibits choline esterase in the blood circulation, but not in the brain.

Conclusions: Monolayer vesicles prepared from novel bolaamphiphiles with acetylcholine head groups can efficiently encapsulate proteins and deliver them across biological barriers such as the cell membrane and the blood-brain barrier.

Keywords: delivery of proteins, bolaamphiphilic vesicles, targeted brain delivery.

* This abstract presents results from the MSc research by Ibrahim Abu Hammad

P-10

DELIVERY OF ANALGESIC PEPTIDES TO THE BRAIN BY NANO-SIZED VESICLES MADE OF MONOLAYERED MEMBRANES

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Lipid-based drug delivery is one of the most popular strategies for increasing drug efficacy and reducing toxicity. Liposomes are among the most extensively studied lipid-based drug delivery systems, but targeting liposomes to specific tissues is still problematic because of stability, ability to cross biological barriers and to release the encapsulated material in a controlled manner. Here we describe the development of monolayer membrane vesicles made of synthetic bolalipids that contain two hydrophilic head groups at each end of a hydrophobic alkyl chain. The concept is to mimic the high chemical and physical stability that characterize membranes of archaebacteria, made from natural bolaamphiphiles. Since it is hard to isolate bolalipids from archaebacteria and their synthesis is complicated, a different approach was taken - synthesizing novel bolaamphiphiles from a naturally epoxidized oil obtained from the plant *Vernonia galamensis*. The novel bolalipids contain acetylcholine (ACh) head groups, which provide the vesicles that they form with a cationic surface. Cationic surfaces promote transcytosis, and thus, enhance the transport of the vesicles via biological barriers. In addition, the ACh head groups may be hydrolyzed by the enzyme acetycholinesterase (AChE), and hydrolysis of the head groups results in the decapsulation of the vesicles and the release of the encapsulated material in tissues that contain AChE (e.g. the brain).

Two similar bolalipids that differ in the way the ACh head group is covalently bound to the alkyl chain were used for obtaining spherical vesicles of 100-200 nm in diameter. In this study we examined if the vesicles can carry peptides with biological activity across the blood brain barrier and exert pharmacological effect within the brain. For this purpose, the vesicles were loaded with the opioid peptide, leu-enkephalin that normally is not available to the brain following its systemic administration. Injection of leu-enkephalin encapsulated within the vesicles with the head groups hydrolyzed by cholinesterases (ChE) into mice that were pretreated with pyridostigmine (to inhibit peripheral ChE) resulted in a significant analgesia. By comparison, injection of non-encapsulated leu-enkephalin or leu-enkephalin encapsulated within bilayer membrane liposomes made from DSPC (neutral liposomes) or DOTAP (cationic liposomes) did not induce significant analgesic effects. In addition, injection of leu-enkephalin encapsulated in vesicles containing head groups that are not hydrolyzed by AChE into pyridostigmine-pretreated mice caused significantly lower analgesic effect than that observed after the injection of vesicles made from bolalipids with head groups that are hydrolyzed by ChE. These results suggest that the novel cationic vesicles are capable of crossing the BBB and release the encapsulated peptide in the brain by the action of brain ChE. Injection of vesicles made from a mixture of both bolalipids with encapsulated leu-enkephalin induced analgesia that lasted longer than that obtained after the injection of vesicles made from a single bolalipid with head groups that are hydrolyzed by ChE. The longer duration of the analgesic effect obtained with vesicles made from a mixture of bolalipids is probably due to a slower decapsulation of these vesicles.

Keywords: bolalipids, vesicles, BBB, controlled release, enkephalin

* The abstract describes results from Maria Popov's PhD thesis.

P-11

FORMATION OF ORGANIC NANOPARTICLES FROM MICROEMULSIONS

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A new method for preparation of hydrophobic organic drug nanoparticles, in a form of water- dispersible powder will be demonstrated for celecoxib. The described method is based on rapid conversion of nanodroplets into nanoparticles, by evaporation of all the solvents from oil-in-water microemulsion containing a dissolved drug in the dispersed volatile oil phase. This process does not require any special instrumentation or high energy investment since the nanodroplets are formed by spontaneous assembly. The microemulsion contains only pharmaceutically approved surfactants. Evaporation is usually carried out by a spray drying. Typically, the process leads to formation of powders composed of either amorphous or crystalline particles in the size range of 10-100 nm[1-3], while in the case of celecoxib amorphous particles with the average size of 17nm were obtained (as verified by DLS and cryoTEM). Dispersing of the powder in water yields a transparent, stable system. The dissolution of the drug is enhanced when the powder is introduced to the aqueous medium. The simplicity and the low cost of the new process make it very attractive for application in the pharmaceutical industry.

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Keywords: celecoxib, nanoparticles, microemulsions

P-12

ROUTE OF ADMINIDTRATION DEPENDENT ANTI-INFLAMMATORY EFFECT OF LIPOSOMAL ALENDRONATE

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Innate immunity and inflammation are of major importance in various pathological conditions. Intravenous (IV) and intraperitoneal (IP) liposomal alendronate (LA) treatments have been shown to deplete circulating monocytes and peritoneal macrophages resulting in the inhibition of restenosis (re-obstruction of the arteries following angioplasty) and endometriosis (EM, outgrowth of endometrial tissue in ectopic tissues), respectively. The aim of the present work was to determine the in vivo biodistribution of LA in healthy and in inflammatory–associated disorders, and to correlate the biodistribution to the extent of monocytes depletion, and to the resultant bioactivity in restenosis and EM. In this work we examined, in two macrophage-dependent inflammatory disorders, the relationships between LA biodistribution on bioactivity following IV and IP administrations. The comparison between IP and IV administrations made it possible to elucidate whether the governing mechanism of drug action was local (depletion of peritoneal macrophages) or systemic (depletion of circulating monocytes). We found that, LA treatment resulted in a dose-response modified biodistribution following both IV and IP administrations. The biodistribution of high-dose LA (10 mg/kg), but not that of the low-dose (1 mg/kg), was similar in healthy and diseased animals. It is concluded that LA impedes its own elimination from the circulation by depleting circulating monocytes and/or inhibiting their endocytic activity, in a dose-dependent manner. Both IV and IP administration of LA mediated by the partial and transient depletion of circulating monocytes effected inhibition of restenosis. Inhibition of EM was effected only by IP administration, which depleted both intraperitoneal and circulating monocytes. Thus, EM should be considered as a local inflammatory condition with systemic manifestations as opposed to restenosis, a systemic inflammatory disease.

Acknowledgment: This research was supported by the Israel Science Foundation (ISF, grant No. 10/09), and by Biorest, Israel.

Keywords: Liposomes; Alendronate; Inflammation; Biodistribution; Monocytes

* Student's research work

P-13

In *VIVO* DELIVERY OF GENES INTO ANTIGEN-PRESENTING CELLS BY MANNOSYLATED POLYION COMPLEXES

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Background: Antigen-presenting cells (APCs) have unique properties in initiating primary immune responses and thus can be an excellent target for DNA vaccination. The dendritic cells' low numbers in peripheral tissues (0.5–2%) considerably limit the efficient vaccination with nontargeted delivery systems. Polyion complex made of poly(ethylene glycol)-*block*-poly(ethylene imine) (PEG-*b*-PEI) conjugates are known to self assemble in aqueous media with DNA into a spherical polymeric, which reduces non-specific interactions with blood components. However, the PEG shielding lowers the transfection efficiency. The attachment of a specific ligand may increase cellular uptake via receptor-mediated endocytosis. The mannose receptor (ManR) involved in the uptake of particulate and soluble antigenic products of bacterial origin represents therefore a promising receptor for targeting APCs having ManR.
Methods: Amino-terminated mannose (Man) ligand, in a mono- (Man–PAP) and tri- (Man_3-AHT) valency were first conjugated with PEG (3500Da) via the N-hydroxy succinimide (NHS) activated terminal. PEI (25000Da) was then conjugated to the mannosylated PEG via the Maleimide (MAL) activated terminal. The resulted positively charged di-block copolymers bearing mannose (Man-PEG-*b*-PEI and Man_3-PEG-*b*-PEI) self-assembled with DNA to form polymer/DNA polyplexes. The ζ-potential and particle size were determined by laser Doppler-anemometry and Dynamic light scattering, respectively.
Results: Man-PEG-*b*-PEI and Man3-PEG-*b*-PEI showed good in vitro complexation with DNA at N/P ratios > 4, and had lower surface charge relative to their PEI building block.. Man_3-PEG-*b*-PEI demonstrated a 3-4 fold greater transfection efficiency relative to Man-PEG-*b*-PEI in ManR positive dendritic cell lines (THP-1, DC2.4). Furthermore, the mannosylated block copolymers demonstrated reduced transfection efficiency in a free Man competition assay, had low cytotoxicity when compared with PEI and showed low transfection efficiency in HeLa cells. Finally, 24h after subcutaneous injection, Man_3-PEG-*b*-PEI demonstrated 2-3 fold greater transfection efficiency relative to Man-PEG-PEI and PEI, in DCs hosted at the lymph nodes of C57/BL6 mice (Figure 1).
Conclusions: These results suggest that the mannosylated block copolymer micelles are an effective gene delivery system for APCs.

Keywords: gene delivery, polyion complexes, dendritic cells, mannose,

*The abstract describes a student's research work

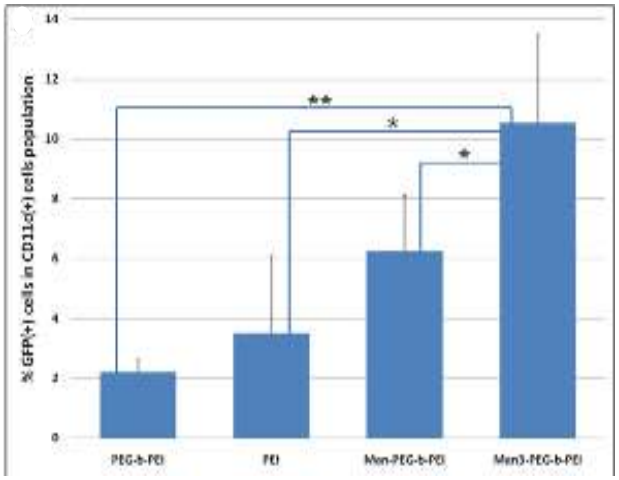

Figure 1 - In vivo uptake of polyplexes by DCs in the draining lymph nodes 24 h after s.c. injection in mice. Polyplexes were prepared with PEI/DNA, PEG-*b*-PEI/DNA, Man-PEG-*b*-PEI/DNA and Man_3-PEG-*b*-PEI/DNA at N/P=8. The percentage of GFP(+) cells in the CD11c(+) cells population was determined by FACS analysis. Values are given as mean ± S.D of three independent experiments. *P<0.05, **p<0.01, ***P<0.001 ANOVA.

P-14

POLYMER BASED DELIVERY SYSTEM OF SIRNA FOR IMMUNOMODULATION

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ABSTRACT SUMMARY

The goal of this study is to design PLGA microspheres (MS) encapsulating small interfering RNA (siRNA) and delivering them into antigen presenting cells (APC) to achieve gene specific down regulation and immuno-modulation.

EXPERIMENTAL METHODS

Anti-human CD80 siRNA was purchased from Ambion Inc and labeled with Cy3. PLGA MS were prepared by the double emulsion solvent extraction method (W/O/W). MS were prepared using NaCl, as an additive, arginine as siRNA carrier or with no preparation additive**.** Labeled microspheres were prepared using 6-coumarin. In order to evaluate morphological properties, MS were visualized by Scanning Electron Microscopy (SEM). Particles' size distribution was analyzed using coulter counter. Efficiency of encapsulation was determined by quantifying siRNA concentration using PicoGreen™ assay, after MS were completely dissolved. siRNA stability was assessed by a poly-acryl-amide gel (PAGE) assay. Release profile was determined by suspending MS in PBS and measuring siRNA concentration in supernatants at different time points. For the generation of human macrophages, THP-1 cells have been treated with PMA. Analysis of MS uptake by macrophages was conducted after co-incubation of the cells with MS. Visualization of fluorescently marked siRNA inside the cells was obtained by fluorescence microscopy.

RESULTS AND DISCUSSION

Particles' size distribution ranged 0.5-10μm, suiting the desired size for phagocytosis. MS have a smooth surface and a spherical shape and are finer when prepared with NaCl. Efficiency of encapsulation was improved as well when MS are prepared with NaCl (up to 30%). Adding arginine as a carrier on the other hand did not yield improvement of encapsulation efficiency. Fluorescence microscopy indicated efficient uptake of MS by macrophages and sustained release of siRNA from MS. PAGE assay results indicated that most of the siRNA remained intact when encapsulated, stored within PLGA particles, and extracted.

CONCLUSION

Our research shows that siRNA can be efficiently encapsulated in PLGA MS and be delivered into APCs. In order to achieve the desired immunomodulation, a peptide will be co-entrapped together with the anti CD80 siRNA molecule.

Keywords: PLGA, Microspheres, Immunomodulation, APC, siRNA.

*This work describes a student's research results.

P-15

IMMUNOMODULATION USING PLGA DELIVERY OF SHORT PEPTIDES TO ANTIGEN PRESENTING CELLS

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ABSTRACT SUMMARY

The goal of this study is to design and optimize PLGA microspheres (ms) for the delivery of specific cancer antigen peptides to antigen presenting cells (APC) to achieve cancer vaccination.

EXPERIMENTAL METHODS

PLGA microspheres preparation**:** Microspheres were prepared using the double-emulsion (W/O/W) solvent extraction method. 6-coumarin, 10% Glycerol and NaCl were added where noted. Morphologic Studies: HRSEM was used to evaluate the shape and surface morphology of ms. Size Distribution: Particles' size distribution was analyzed using a Coulter LS 230. In vitro release of GP209-FITC (9 amino acids fragment of the GP100 melanoma associated protein) peptide from PLGA ms: The concentrations of GP209-FITC peptide in the release medium were evaluated using an ELISA reader. Encapsulation efficiency: Encapsulation was determined using ELISA reader after complete degradation of microspheres. Cells**:** Mouse macrophage-like cell line RAW264.7. For the generation of human macrophages, THP-1 cells have been treated with PMA. Microspheres uptake by APCs**:** Cells were co-cultured with fluorescent labeled ms for different time periods and visualized using fluorescent and confocal microscopy and analyzed using FACS. Viability of APCs post ms incubation: Viability was measured using AlamarBlue® up to 14 days post incubation with ms. Peptide presentation: Peptide released from ms and presented on MHC-I was analyzed using specific T cell-like Ab.

RESULTS AND DISCUSSION

Targeting of PLGA microspheres into APCs require that the particles' diameter will be in the range of 1-10um. Analyses of ms preparations showed particles' diameter ranging 1-10 um. HRSEM indicated round, shaped, intact ms, with smooth morphology. Encapsulation efficiencies of GP209 peptide in several preparations and different inherent viscosities were evaluated. NaCl and glycerol improved encapsulation efficiency (up to 45%) and reduced the burst effect. Confocal microscopy indicated that ms are located inside the cells. FACS analyses indicated that the uptake of microspheres is time dependent. Cytochalasin B treated cells did not uptake ms which means ms are up taken by phagocytosis. Viability assays showed no reduction in cells' viability even after 24 hours of co-incubation. No reduction in viability is seen even after 14 days of incubation. Antibodies with T cell like specificity confirmed the integrity of peptide released from ms and its ability to be presented by MHC-I complexes.

CONCLUSION

Our findings demonstrate that PLGA microspheres may serve as antigen delivery system to APC and that peptides released from ms retain their integrity and presentation capability.

Keywords: PLGA, Microspheres, Vaccination, APC, peptides.

* This work describes a student's research results.

P-16

INTRACELLULAR TARGETING OF ANTIGENIC PEPTIDES FOR ENHANCEMENT OF ANTICANCER VACCINATION

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Background: Targeting of drug to its site of action can increase the magnitude of the desired effects and reduce the magnitude of adverse effects that are obtained during drug treatment. One of the targeting approaches is an intracellular targeting. In this way, targeted delivery of antigenic peptide to the organelles involved in the peptide cross-presentation within the antigen presenting cells can lead to efficient mounting of cellular immune response against cancer cells. This targeting approach can improve the efficiency of the existing anti-cancer vaccines that showed limited clinical effects due to inefficient activation of the cytotoxic T lymphocytes directed against the tumor cells.
Aims: 1) Development and characterization of endoplasmic reticulum (ER)-targeted antigen delivery system (ADS). 2) *In vitro* assessment of the antigen delivery system: cellular uptake and intracellular trafficking.
Methods: The experimental system was based on the immunodominant $OVA_{257-264}$ peptide (SIINFEKL) of ovalbumin (OVA) which presentation restricted to the H-$2K^b$ MHC class I heavy chain-β_2m dimers. Antigenic peptide-loaded nanoparticles were prepared using a double emulsion technique and their morphology, ζ-potential, encapsulation efficiency and release rate of the encapsulated material (antigenic peptide) were characterized. The nanoparticle's surface was decorated by conjugation of targeting or control peptides. Efficiency of nanoparticles' intracellular uptake and localization were tested in HeLa cells using fluorescent microscopy.
Results: Particles with desired size (~300 nm diameter) and efficient encapsulation of antigenic peptide were prepared. Biphasic *in vitro* release of the antigenic peptide was observed: burst effect (~30%) followed by gradual release over 3-4 days. The uptake of the nanoparticles into the cells was influenced by the presence and sequence of the conjugated peptides. In addition, the conjugated nanoparticles (with ER-targeting or control peptides) accumulated to a higher extent in the ER and in the endosomes in comparison to the unconjugated nanoparticles.
Conclusions: Conjugation of the targeting residues affects the uptake and intracellular localization of the nanoparticles. More detailed investigation is required to determine the mechanism and suitability of intracellular targeting for the purpose of anticancer vaccination.

Keywords: intracellular targeting, antigenic peptide, anticancer vaccination, nanoparticles.

* This abstract presents results of MSc research by Hadas Sneh and Diana Likhtenshtein

P-17

BETA-CASEIN MICELLES AS ORAL NANO-VEHICLES FOR CHEMOTHERAPEUTIC DRUGS

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Purpose: to entrap a hydrophobic chemotherapeutic drugs, e.g. taxol, in micellar nanoparticles of beta casein (β-CN). Beta-CN is an amphiphilic milk protein, which forms micelles, mainly by hydrophobic interactions. Drug encapsulation in β-CN was performed in order to improve drug solubility in aqueous medium, and achieve targeted oral delivery to the stomach for the treatment of gastric cancer. We studied β-CN-taxol nanoparticles in terms of drug entrapment, colloidal stability, and drug release in the stomach by simulating gastric digestion. Taxol cytotoxicity following simulated digestion was compared to the cytotoxicity of untreated drug on N-87 gastric carcinoma cell lines and on MCF 10A healthy breast epithelium cells.
Methods: Taxol entrapment was performed by stirring a dimethyl sulfoxide (DMSO) solution into a phosphate-buffered saline containing β-CN. Gastric digestion was performed by simulating gastric conditions: pH=2, 37°C, continuously shaking for two hours, using pepsin - a gastric proteolytic digestion enzyme. The cytotoxic activity of taxol after encapsulation in 1mg/ml β-CN at optimal taxol: β-CN molar ratio and simulated gastric digestion at optimal conditions set earlier on N-87 and MCF 10A cells was compared to the cytotoxic activity of untreated taxol.
Results: The optimal molar loading ratio was found to be about 6:1 taxol:β-CN. β-CN-taxol nanoparticles were stable for two weeks without preservatives. 70% of the taxol remained entrapped in β-CN nanoparticles for two weeks. The optimal pepsin: β-CN w/w ratio for simulated gastric digestion experiment was 50:1. Beta casein was digested in 10 minutes and 80% of the drug was released during this period. No further release was observed up to 2hrs of simulated gastric digestion. After encapsulation at 6:1 taxol: β-CN molar ratio and simulated digestion at optimal conditions: 50:1 β-CN: pepsin w/w ratio, at 37°C, continuously shaking during 20 min Taxol didn't lose its cytotoxic activity. Its drug concentrations required to inhibit cell growth by 50% (IC_{50}) values were slightly higher (32.5±6.2 and 17.8±1.4 for N-87 and MCF 10A cells respectively) but similar to that of untreated taxol (25.4±2.6 and 12.0±2.1 for N-87 and MCF 10A cells respectively).
Conclutions: Beta-CN forms colloidally-stable nanovehicles of hydrophobic anticancer drugs; it is easily digested under simulated gastric digestion conditions and may be used for oral-delivery of chemotherapeutics.

Keywords: β-casein micelles; targeted oral-delivery; gastrointestinal tract; cancer; hydrophobic chemotherapeutics; cell cytotoxicity; taxol; N-87; MCF 10A.

*The abstract describes Alina Shapira's PhD research results.

P-18

PREVENTION AND TREATMENT OF ORAL SQUAMOUS CELL CARCINOMA USING SIROLIMUS SUSTAINED RELEASE DELIVERY SYSTEMS FOR LOCAL APPLICATION

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Oral squamous cell carcinoma (OSCC) is an oral malignant disease characterized by a grim prognosis. Currently, modern medicine has no response for preventing OSCC at early stages and clinicians define the situation as "watchful waiting". The patients simply wait for cancer to develop and then chemotherapy and surgical intervention are needed. Thus, a new chemoprevention approach is needed.

Sirolimus, a potent mTOR pathway blocker, was recently reported to be very effective for the prevention and treatment of the OSCC. Unfortunately, the severe adverse effects of the drug prohibit its usage as a preventive treatment.

Topical sustained release (SR) drug delivery systems (DDS), alongside with targeting the drug have the advantages of reducing the required dose; prolong its duration in the oral cavity, thus enhancing its therapeutic potential, while reducing its side effects.

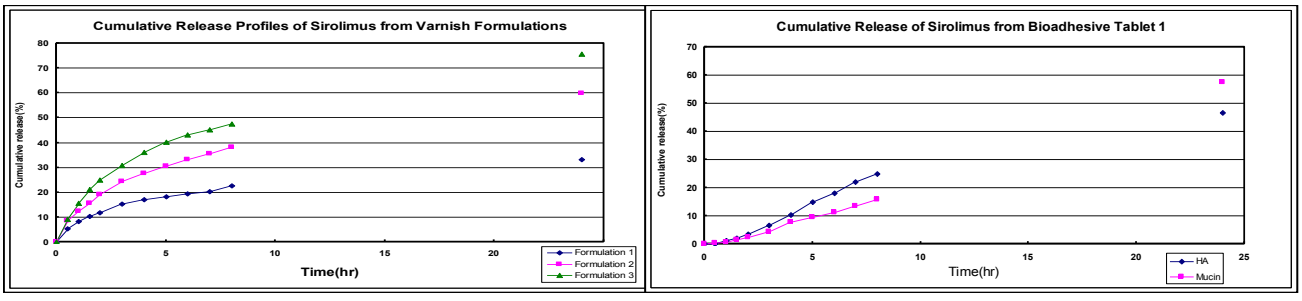
The aim of the research was to develop novel polymeric sustained release (SR) formulations of an anti-proliferative agent sirolimus and to evaluate its release profiles in vitro.

Several nondegradable hydrophobic polymer based varnish formulations and hydrophilic based bioadhesive tablets containing sirolimus were prepared and active ingredient release profiles were evaluated.

All formulations exhibited variable SR profiles according to the set goals of this study:

The use of nondegradable hydrophobic SR varnish formulations containing sirolimus were shown to release 33% to 75% of its sirolimus content within 24 hours.

Hydrophilic polymer based bioadhesive tablets containing sirolimus were shown to release 46% to 58% of its sirolimus content within 24 hours.



These results suggest that the release of sirolimus can be prolonged utilizing different polymeric formulations.

Keywords: squamous cell carcinoma, sirolimus (rapamycin), sustained release topical delivery system.

P-19

CONTROLLED DELIVERY SYSTEM TO ELICIT ANTI-CANCER EFFECTS OF HIV PROTEASE INHIBITOR NELFINAVIR

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Nelfinavir is an HIV-protease inhibitor that has been used to treat AIDS patients since 1993. Nelfinavir was found to possess anti-cancerous properties due to its ability to inhibit Akt phosphorylation, which is a crucial step in cancer cells' survival and growth, and/or resistance to anti-cancer therapy. The main problem with Nelfinavir treatment is that Akt has major regulatory roles also in non-cancerous cells, which may result in severe side-effects. In addition, it was found that increasing Nelfinavir's concentration does not further inhibit tumor growth compared to lower doses, suggesting that prolonged exposure to Nelfinavir is more crucial for effective treatment than higher dosage. Therefore a tumor-targeted controlled-delivery system for Nelfinavir is required to allow prolonged exposure to effective drug concentrations.

In the current study, Nelfinavir is entrapped in a polymeric matrix of

Poly (lactic-co-glycolic)acid (PLGA). The polymer dissolved in glycofurol solidifies upon contact with aqueous media and may be injected directly to the tumor or its immediate environment. The polymer degrades at a known rate maintaining an effective concentration of the drug in the tumor.

Three phases in the release kinetics were observed: the burst phase – rapid release in the first 20 minutes due to incomplete implant solidification, the diffusion phase – (first 10 hours) of the drug diffusing from the implant's surface, where the release rate can be set by changing the loaded amount of the drug, and the very slow release of the drug entrapped in the implant as the implant slowly degrades. The effect of controlled release system of Nelfinavir was tested *in-vitro* using MCF-7 breast cancer cell line for viability, proliferation and Akt phosphorylation in a tumor-like environment. The cells' viability decreased gradually and reached 30% of control within 96 hours of exposure to Nelfinavir. The cells' proliferation was completely inhibited by Nelfinavir and Akt phosporylation inhibition appears to be the mechanism of action responsible for Nelfinavir's toxicity. This conclusion is further supported by the toxic effect of Nelfinavir's controlled release system on viability of B-16 melanoma cell line with constitutivly active Akt.

These results clearly show that a controlled delivery system for Nelfinavir is effective in both of the *in-vitro* models which show promise for its *in-vivo* effectiveness as well.

Keywords: Nelfinavir, cancer, drug delivery, polymer.

*This abstract describes a student's research results.

P-20

TAILORING DELIVERY SYSTEMS FOR GLIOMA CANCER CELL THERAPY

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INTRODUCTION

Glioblastoma is characterized by a migration of tumor cells into normal brain parenchyma, extensive vascularization and high proliferation rate of endothelial and tumor cells. Our aim is to develop a new treatment that will be based on local delivery of encapsulated anti-cancer drugs (S-TRAIL, AZD2171, SB431542), which operate, by different mechanisms using PLGA microspheres. Our future goal is to target not only glioma cells but also glioma stem cells that have ability of self-renewal and multipotential differentiation.

METHODS

PLGA microspheres were prepared using solvent-evaporation technique with some modification. Size distribution of the PLGA microspheres was determined by a coulter counter. Release kinetics of s-Trail, AZD2171, SB431542 was studied using Elisa, HPLC, and spectrophotometer detection assays respectively. The biological activity of the released and pure s-TRAIL, AZD2171 and SB431542 was studied on the human U87and A172 cell lines. Viability and proliferation of the cells was measured by AlamarBlue and thymidin assays, respectively. FACS analysis were be used to detect the level of cell apoptosis and necrosis.

RESULTS

The release profiles of the encapsulated drugs demonstrated initial burst during the first 3 days and then had a continuous release. Microspheres with diameters of 20-80μm represent most of the volume as determined by the light scattering method**.** Pure and released S-Trail, AZD2171 and SB431542 led to a decrease in cell viability and proliferation in a dose–dependent manner, yet the cytotoxic effect of AZD2171 was much more significant.

CONCLUSIONS

1. The PLGA microspheres loaded with s-Trail demonstrated a sustained cytotoxic effect for more than 20 days on U87 cells.
2. The PLGA microspheres loaded with AZD2171 and SB431542 showed a cytotoxic effect after 5 day treatment on U87 and A172 cells.
3. AZD2171 seems to be a promising agent for local delivery since it led to a significant decrease in cell viability and proliferation of U87 and A172 cell lins.

Keywords: PLGA, Glioblastoma, s-trail, AZD2171, SB431542.

*The abstract describes a student's research results

P-21

ULTRASOUND GENE DELIVERY TO MESENCHYMAL STEM CELLS: TARGETING TUMOR THERAPY

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Background

Gene therapy of 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 are emerging as substitutes to the viral ones since they are considered safer, easier to prepare, lack immunogenic response, and do not have a limit in the size of gene introduced.

Methods

TUS-transfection in-vitro was assayed on MSC. pLuciferase and pGFP with and without ultrasound contrast agents (USCA) were used to study transfection level and efficiency respectively. 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 in the cells and on the nucleus was also studied.

Results

TUS of 2W/cm^2, 30% DC operated for 20 or 30 min led to the transfection of MSC, when using 10 or 12 μg/ml of cDNA. Adding USCA resulted in higher transfection efficiency. TUS did not affect significantly the viability of the cells and did not alter the stemness of the cells. In-situ Time-laps imaging revealed that during TUS application the nucleus undergoes reversible morphological changes.

Conclusions

Our study demonstrates that TUS can efficiently transfect MSC while maintaining their viability. 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.

* The abstract describes a student's research results

P-22

IN VIVO DELIVERY OF SIRNA TO TUMORS AND THEIR VASCULATURE BY NOVEL DENDRITIC NANOCARRIERS

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Background: New targets for RNAi-based cancer therapy are constantly emerging. Nevertheless, *in vivo* delivery of siRNA remains a crucial issue for its therapeutic success. We propose to encapsulate the siRNA in a cationic carrier system, which can strongly improve its stability, cellular uptake and silencing efficacy. We developed novel polymerized dendrimer core shell structures to deliver siRNA *in vivo*. These water-soluble macromolecular carriers accumulate in the tumor environment due to the enhanced permeability and retention (EPR) effect and therefore, represent ideal delivery vehicles for antitumor biological agents.

Methods: Dendritic nanocarriers were synthesized and further characterized by dynamic light scattering, atomic force microscopy and electrophoretic mobility shift assay. Cellular internalization of polyplexes was monitored by confocal microscopy. The nanocarriers cytotoxicity and biocompatibility profiles were assessed by XTT and red blood cells lysis assay. The luciferase gene, ectopically overexpressed in human glioblastoma cell lines was used as a model system and its silencing efficacy was measured. mCherry and luciferase-labeled glioblastoma and mammary adenocarcinoma mouse models were established. The silencing efficiency of the nanocarrier luciferase-siRNA polyplexes was followed up by non-invasive intravital bioluminescence imaging.

Results: All dendritic nanocarriers synthesized had a mean hydrodynamic diameter of 10-40 nm. The novel nanocarriers entrap siRNA, neutralize its negative charge in a dose-dependent manner and significantly improve its cellular uptake. Polyglycerol-amine dendrimers (PG-Amine) exhibited the optimal silencing efficiency and safety profile in additional *in vitro* biocompatibility and efficacy tests. Therefore, it was selected for further evaluation and *in vivo* gene silencing efficacy studies. A significant gene silencing effect was accomplished *in vivo* in both human glioblastoma and murine mammary adenocarcinoma mouse models. Within 24 hours, 85% and 68% silencing was achieved following intratumoral and intravenous treatment respectively, as measured by intravital non-invasive imaging of photon flux bioluminescence. No significant weight loss occurred following intravenous administration of the siRNA-nanocarrier complexes.

Conclusions: We show a proof of concept for siRNA delivery using a luciferase-based model. We predict that *in vivo* silencing of an important cell growth and angiogenesis regulator as Akt1 in a selective manner will warrant this approach as a successful anticancer therapy.

Acknowledgement: This research was supported by the Israel Science Foundation, the Marguerite Stolz Research Fellowship Fund for Outstanding Junior Faculty, the Adams Super Center for Brain Studies and the Joan and Jame Constantiner Institute for Molecular Genetics.

Keywords: small interference RNA, polymer therapeutics, dendrimers, luciferase, glioblastoma.

P-23

TARGETED POLYMER THERAPEUTICS BEARING PACLITAXEL FOR ANTI-ANGIOGENIC AND ANTICANCER ACIVITY

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Angiogenesis, new capillary blood vessel growth from pre-existing vasculature, is a critical factor in cancer progression. Therefore, anti-angiogenic therapy, alone or in combination with conventional cytotoxic therapy, may be a promising therapeutic approach. Paclitaxel (PTX) is a potent cytotoxic hydrophobic drug that exhibits anti-angiogenic effects at low dose; however, its use is limited by severe side effects. Nanoconjugates of polymers with anticancer drugs passively target the tumor tissue due to the enhanced permeability and retention (EPR) effect while extravasating via the tumor leaky neovasculature. Here, we designed and synthesized a polyglutamic acid (PGA)–PTX-E-[c(RGDfK)$_2$] conjugate. We hypothesized that a combination of a PGA-PTX conjugate containing cyclic RGD peptidomimetic (PM) might enhance the effects seen for PGA-PTX alone. This hypothesis is based on the additional active targeting to the $\alpha_v\beta_3$ integrin overexpressed on proliferating tumor endothelial cells and several types of tumor cells. This multidisciplinary approach should offer a higher therapeutic efficiency with reduced side-effects.

Paclitaxel was designed to be released from PGA under lysosomal acidic pH, while the PGA is enzymatically degradable. PGA-PTX-E-[c(RGDfK)$_2$] demonstrated anti-angiogenic activity by inhibiting the proliferation of endothelial cells, their migration towards vascular endothelial growth factor (VEGF), their capillary-like tube formation and their attachment to fibrinogen, which was not affected by PGA-PTX-c(RADfK) control conjugate. PGA-PTX-E-[c(RGDfK)$_2$] conjugate inhibited the proliferation of pancreatic adenocarcinoma cells and to a higher extent $\alpha_v\beta_3$-expressing glioblastoma cells and breast cancer cells. For PTX-resistant human breast cancer cells, the conjugate was able to overcome the resistance, indicating an advantage for the conjugate over free PTX. In addition, PGA-PTX-E-[c(RGDfK)$_2$] was able to disturb the 3D glandular formation of $\alpha_v\beta_3$-expressing human breast cancer cells to a larger extent than free PTX. Preferential tumor accumulation of PGA-PTX-E-[c(RGDfK)$_2$] conjugate was seen compared with the control PGA-PTX-c(RADfK) conjugate. *In vivo* study demonstrated the advantage in safety of the PGA-PTX-E-[c(RGDfK)$_2$] over PTX, when mice treated with low concentration of 4 mg/kg/q.o.d. PTX showed signs of toxicity, such as weight loss, whereas conjugate-treated mice gained weight. Our preliminary results using PGA-PTX-E-[c(RGDfK)$_2$] conjugate support our strategy as a novel targeted bi-specific anti-angiogenic and anticancer therapy.

Keywords: Angiogenesis, polymer therapeutics, paclitaxel, $\alpha_v\beta_3$ integrin, RGD, polyglutamic acid

P-24

MULTIFUNCTIONAL ARCHITUCTURES OF POLYMER THERAPEUTICS DELIVERING PACLITAXEL AND ALENDRONATE TO BREAST CANCER BONE METASTASES

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Paclitaxel (PTX), is one of the most effective chemotherapeutics drugs used for the treatment of breast cancer bone metastases. In recent years, it has become evident that PTX at low doses inhibits angiogenesis. Despite its strong anticancer activity, PTX exhibits serious dose-limiting toxicities due to the absence of selectivity for target tissue. In addition, because PTX is water insoluble, it is administered in Cremophor EL vehicle, which causes severe allergic, and hypersensitivity reactions.

To address these problems, a variety of delivery systems are being investigated to administer PTX in a safer and convenient manner. In addition, a specific targeting molecule could be combined with multivalent polymers. We designed and synthesized two polymer conjugates of PTX and ALN. Both drugs were conjugated with *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer and with poly(ethylene glycol) (PEG) polymer. ALN will facilitate the delivery of PTX to the bones. The conjugation with a polymer would target PTX mostly to the metastatic sites within the bones and scarcely to normal healthy bones due to its passive extravasation through the leaky tumor vessels. We have previously reported the conjugation of PTX with HPMA copolymer-Gly-Phe-Leu-Gly-*p*-nitrophenol (HPMA copolymer-GFLG-ONp) through Phe-Lys-*p*-aminobenzyl carbonate (FK-PABC) spacer (K. Miller et al., *Angewandte Chemie International Edition* 48, 2949 –2954 (2009). This dipeptide-PABC linker provided a stable conjugation chemistry of PTX with HPMA copolymer by a carbonate linkage. Both GFLG and FK linkers were cleaved by the lysosomal enzyme cathepsin B. We now report the design and synthesis of a second conjugate composed of PEG-PTX-ALN micelles. PTX was bound to PEG polymer by an ester bond and four molecules of ALN, which were conjugated through a 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. The physicochemical properties, biocompatibility, anticancer and anti-angiogenic activity of both HPMA-PTX-ALN and PEG-PTX-ALN conjugates were analyzed *in vitro*. The anticancer and anti-angiogenic activity of the conjugates on breast cancer bone metastases mouse model was evaluated using non-invasive intravital imaging. Both conjugates were non-toxic and exhibited better efficacy by inhibiting tumor growth in the bones as compared to the combination of the free PTX and ALN. A comparison between the two architectures, micelles *versus* hyperbranched polymer conjugate, was accomplished.

The described data warrants the potential use of PEG-PTX-ALN and HPMA-PTX-ALN as a novel bone targeted anticancer and anti-angiogenic therapy for breast cancer bone metastases.

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* The abstract describes a student's research results.

P-25

PEG-DOX-E-[c(RGDfK)$_2$] CONJUGATE FOR TARGETING INTEGRINS ON CANCER CELLS AND TUMOR VASCULATURE

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Doxorubicin (DOX) is extensively used in cancer therapy; however, it is cardiotoxic in cumulative doses and chemoresistance can evolve with its prolonged use. Conjugation of a chemotherapeutic agent with a water-soluble polymeric carrier prolongs the circulation life of the drug, promotes its accumulation at the tumor site due to the enhanced permeability and retention (EPR) effect and prevents the drug from extravasating into healthy tissues.
We designed and synthesized a delivery system that enables the conjugation of a targeting moiety, cyclic Arg-Gly-Asp (RGD) peptidomimetic, on one end of a linear poly(ethylene glycol) (PEG) chain, and DOX on the other end. The drug was bound to the polymer through an acid-sensitive (6-maleimidocaproyl)hydrazone linker. The resulting PEG-DOX-E-[c(RGDfK)$_2$] conjugate actively and selectively targets endothelial and tumor cells overexpressing $\alpha_v\beta_3$ integrin. We hypothesized that this conjugation will result in a potential treatment which will selectively accumulate in the tumor vascular bed and inhibit specifically and effectively tumor growth.
The PEGylation of DOX and E-[c(RGDfK)$_2$] resulted in a conjugate of 13 kDa in size. To evaluate the interaction between E-[c(RGDfK)$_2$] and the $\alpha_v\beta_3$ integrin receptor, we first determined the expression levels of the $\alpha_v\beta_3$ integrin in several cell types using fluorescence-activated cell sorting (FACS). The fluorescent properties of doxorubicin were utilized to follow the cellular uptake of PEG-DOX-E-[c(RGDfK)$_2$]. It was found that PEG-DOX-E-[c(RGDfK)$_2$] conjugate binds and internalizes into U87-MG glioblastoma cells overexpressing $\alpha_v\beta_3$ integrin. The anti-angiogenic properties of our conjugate were evaluated on human umbilical vein endothelial cells (HUVEC). *In vitro* cytotoxicity assay demonstrated a similar cytotoxic effect of the novel nanoconjugate as free DOX. In addition, PEG-DOX-E-[c(RGDfK)$_2$] had an inhibitory effect of ~75% on HUVEC attachment to fibrinogen. Preliminary *in vivo* near-infrared studies revealed that a PEG-E-[c(RGDfK)$_2$]-cyanine conjugate preferentially accumulated in mCherry-labeled-DA3 murine mammary tumors inoculated in mice compared with a non-targeted or a control RAD-bound conjugate.
By showing the advantages of our conjugate which accumulates selectively at the tumor site, we hope to warrant it as a novel targeted, anti-angiogenic and anticancer therapy.

Keywords: Tumor angiogenesis; $\alpha_v\beta_3$ integrin; polymer therapeutics; doxorubicin; poly(ethylene glycol)

*The abstract describes a student's research results.

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TARGETING THE ANGIOGENIC SWITCH OF CALCIFIED TUMORS USING RAFT-SYNTHESIZED NANOCONJUGATES

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There is an unmet medical need for new targeted treatments for bone neoplasms such as osteosarcoma and bone metastases. Over the past two decades, multi-modality treatment consisting of aggressive chemotherapy combined with radical surgical resection, has been the mainstay of bone neoplasms management. However, still one-third of the patients die from bony tumors, and for those with unresectable disease there are no curative systemic therapies.

Angiogenesis is a major regulator of tumor progression and bone metastases, consequently, angiogenesis inhibitors are emerging as a new modality for anticancer therapy. We developed a new therapeutic strategy to target bone metastases and calcified neoplasms using combined polymer-bound angiogenesis inhibitors. Using an advanced living polymerization technique (RAFT-Reversible Addition-Fragmentation chain Transfer), we conjugated the aminobisphosphonate alendronate (ALN), and the potent anti-angiogenic agent TNP-470 with N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer through a Gly-Gly-Pro-Nle linker, cleaved by cathepsin K (E. Segal et al., PLoS One 4, e5233, 2009). We show that a combination of ALN plus TNP-470 has synergistic anti-angiogenic activity on the inhibition of proliferation of endothelial cells *in vitro*. Free and polymer-conjugated ALN-TNP-470 showed anti-angiogenic and antitumor activity by inhibiting the *in vitro* proliferation, migration and capillary-like tube formation of endothelial cells and proliferation of human osteosarcoma cells. Our conjugate reduced vascular endothelial growth factor (VEGF)-induced vascular hyperpermeability by 92% and remarkably inhibited osteosarcoma growth in mice by 96% compared with 45% inhibition by the non-conjugated ALN plus TNP-470.

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.

Acknowledgements: This work was supported by THE ISRAEL SCIENCE FOUNDATION (1300/06) (RSF), the Israel Cancer Association, the Israel Cancer Research Foundation (RSF) and NIH grant RO1 GM069847 (JK). We thank the TAU Center for Nanoscience and nanotechnology, The European Association for Cancer Research and the Shtacher Family Fellowship for their financial support (ES).

Keywords: Angiogenesis, calcified tumors, polymer therapeutics.

*The abstract describes a student's research results.

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NANOLIPOSOMAL DRUGS COMBINED WITH THERMAL ABLATION IN ANTI-CANCER THERAPY

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We hypothesized that by using two types of anticancer therapy simultaneously we will obtain a significantly more effective therapy than from each alone.

We used two different nanoliposomal drugs: LipoViTo and Doxil (injected into to the tail vein). Both were used with radiofrequency thermal ablation (RF) – an anticancer therapy based on in-situ tumor heating.

As an animal model was used NUDE-Hsd: athymic mice with human cancers with two types of cancers were used: lung cancer (cell lines A459) and medulloblastoma (cell line Daoy) for injected subcutaneously onto the back of mice. After 4-5 weeks, the size of tumors was about 14-15 mm, and optimal the experiment.

In the first experiment we wanted to find the optimal regime of therapy.

For it we used mice with medulloblastoma LipoViTo and RF. The groups were: (1) RF and then LipoViTo after 15 min; (2) LipoViTo and RF after 15 min; (3) LipoViTo and RF after

24 h; (4) first, RF and then LipoViTo after 24 h; (5) RF alone; (6) LipoViTo alone and (7) control (no treatment). After 48 h from the beginning of treatment, mice were sacrificed, tumors were excised, sliced to 2-3 mm thick disks, stained in tetrazolium chloride for ~10 min, and area of necrosis was measured by electronic caliper.

Results: in groups 1-4, the necrosis area was significantly larger than in groups 5-7, and in group 1, it was maximal.

In the second experiment we used RF and then drug (LipoViTo or Doxil) after 15 min, for both cancers. Groups for each type of cancer were: (1) RF and LipoViTo after 15 min; (2) RF and Doxil after 15 min; (3) LipoViTo alone; (4) Doxil alone; (5) RF alone; and (6) control. Preparation of sample was the same as in the first experiment.

Results: in medulloblastoma, the combination of each drug with RF worked significantly better than each drug alone or RF alone or control. In lung cancer only Doxil + RF was significantly better than other groups.

Conclusions: simultaneous RF and drug therapy is better in both types of cancer than is RF or drug therapy alone. A difference in line response suggests that organ-specific. Factoring at therapy will likely be necessary.

Keywords: Doxil, LipoViTo, Radiofrequency, Cancer.

P-28

E-SELECTIN TARGETED POLYMER CONJUGATES FOR TREATMENT OF PRIMARY TUMORS AND LUNG METASTASIS

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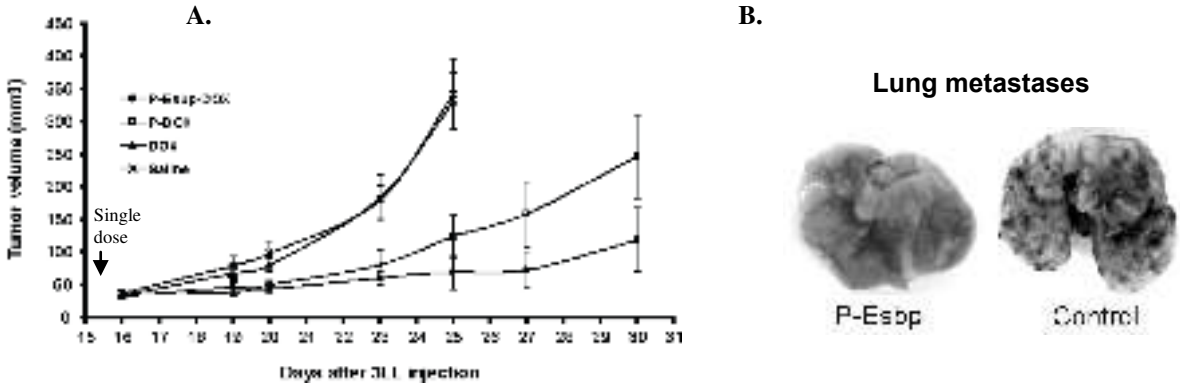
The site-specific expression of E-selectin on tumor endothelium and its involvement in metastasis of cancer provides an opportunity to target drugs to both primary tumors and their metastatic lesions. We hypothesized that synthetic *N*-(2-hydroxypropyl)methacrylamide (HPMA) polymeric carriers presenting a multivalent display of E-selectin binding peptide (Esbp, sequence DITWDQLWDLMK) will promote the accumulation of the conjugates in tumor vasculature or the microenvironment of metastatic tumor cells by virtue of the specific recognition by E-selectin and the enhanced permeability and retention (EPR) effect. Multiple copies of Esbp peptide were efficiently coupled to HPMA copolymer precursor using the native chemical ligation method, to give P-Esbp conjugate. Then, Doxorubicin was covalently linked to P-Esbp conjugate via pH sensitive hydrazone bond (P-Esbp-DOX). Doxorubicin containing copolymer conjugates were evaluated for their *in vitro* and *in vivo* cytotoxicity. For evaluation of anti-tumor activity *in vivo*, conjugates were injected intravenously to C57BL/6 mice bearing murine Lewis Lung Carcinoma (3LL) tumors. To examine the ability of Esbp-copolymer conjugates (P-Esbp, without a drug) to inhibit cancer cell lung colonization, $1X10^6$ B16F10 melanoma cells were injected intravenously (IV) 45min after IV injection of P-Esbp and the formation of pulmonary metastases was analyzed 2 weeks post injection.
Our results show that E-selectin targeted copolymers were bound to surface-associated E-selectin *in vitro* with affinity at the low nano-molar range, and their cytotoxicity towards E-selectin expressing immortilized vescular endothelial cells (IVEC) was 150 folds greater than that of non targeted P-DOX. A single dose of P-Esbp-DOX significantly reduced tumor size (Figure A) and prolonged mice survival with no apparent adverse toxicity. Moreover, P-Esbp conjugate completely inhibited the lung colonization of B16F10 melanoma cells and prevented the formation metastatic colonies (Figure B). This data indicates that E-selectin can serve as a useful target for cancer therapy.



A) E-selectin targeted HPMA copolymer DOX conjugate **enhanced tumor growth inhibition** compared to non-targeted HPMA copolymer-bound DOX. **B)** Complete ihibition of lung metastases in mice treated with E-seletin targeted HPMA copolymer.

Keywords: HPMA conjugate, Drug delivery, E-selectin, Cancer metastasis

* The abstract describes a student's research work

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POLYMER CONJUGATES FOR VISUALIZING SOLID TUMORS IN THE GASTROINTESTINAL TRACT

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Introduction: Targeting of imaging probes specifically to diseased tissues such as colorectal cancer is attracting because it potentially allows the improvement of tumor detection. One of the problems associated with conventional, low molecular weight imaging probes is the limited tumor to background ratio. To circumvent this, imaging probes (FITC or near infrared fluorescence (NIRF) dye (IR-783-COOH)) were conjugated to HPMA-based polymeric carriers, and their ability to passively accumulate solid tumors of the gastrointestenal (GI) tract was tested. The active targeting through the incorporation of short peptide sequences (GE11 and C3-G12) that mediate binding to cancer-specific antigens (EGFR and galectin-3, respectively) were tested both *in vitro* and *iv vivo*.

Methods: HPMA-based polymer carrying FITC or IR-783 as imaging molecule and C3-G12 or GE11 peptide as targeting moieties were synthesized by radical precipitation copolymerizing. The binding of the polymers into various colorectal cancer cells (CRC) cells was tested by FACS following the incubation of with the targeted polymers. The sub-cellular fate of polymers was also monitored using confocal microscopy. Activation of quenched NIRF probes was tested *in vitro* by fluorescents measurement in presence and absence of lysosomal cystein protease, Cathepsin B. *In vivo* imaging of orthotopic xenograft tumors derived from human adenocarcinoma cells (SW-480) in nude mice was performed by IVIS Lumina Imaging System after intravenous administration of the fluorescent probes.

Results and Conclusions: Polymeric probes carrying C3-G12 or GE11 as targeting ligands demonstrated superior binding affinity to colorectal cancer cells relative to the control, non-targeted probes, followed by internalization and localization at the lysosomal compartments. This confirms the use of short peptides with high binding affinity to galectin-3 or EGFR over-expressing cells to improve the detection of CRC tumors. NIRF imaging probes with increasing molar percentages of dye demonstrated fluorescence quenching and a recovery of the fluorescent signal following the degradation by Cathepsin B. *In vivo* imaging showed passive tumor accumulation of the IR-783 bearing probes 4 hours after intravenous injection and retention at the tumor site for at least 48 hours. These findings indicate that HPMA-based copolymers carrying NIRF imaging probe are an effective tool for tumor detection based on over-expression of proteolytic enzymes involved in tumorigenesis.

Keywords: colorectal cancer, near infrared fluorescence (NIRF), HPMA-based copolymers, Cathepsin B

* This abstract describes a student's work.

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HYALURONAN – POLYMER CONJUGATE FOR SELECTIVE TARGETING PACLITAXEL TO CD44 EXPRESSING CANCER CELLS

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Background: Drugs administered to treat cancers typically become distributed randomly throughout the body, resulting in the arrival of a lower concentration of the drug at the tumor site and some severe side effects due to the lack of specificity of the drug. In order to increase the efficacy of the treatment, a targeted delivery system based on polymer-oligosaccharide conjugates has been designed. The system contained the water-soluble N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer backbone and hyaluronic acid (HA) as a ligand for targeting the CD44 cell-surface receptor, associated with a wide variety of tumors and the metastatic spread of cancer. Recently it was reported that the critical size of HA oligomers needed to exhibit monovalent binding to CD44 is between 6 and 18 monosaccharides. An increase in the binding avidity of HA was seen with oligomers ($\geq HA_{20}$) that can interact with two CD44 molecules simultaneously. Our aim was to design targeted HPMA copolymer conjugates bearing HA oligomers of a specific length (HA_{34} = 34 saccharide units) and to investigate the binding affinity and selectivity in CD44-expressing cells.

Methods: The FITC-labeled HPMA copolymer precursor having active ester groups for HA attachment (designated as P-(GG-ONp)-FITC, where P represents the HPMA copolymer backbone) was synthesized by radical precipitation polymerization of the following monomers: MA-AP-FITC, MA-GG-ONp and HPMA. HA_{34} was conjugated to lysine residue and then coupled with the copolymer precursor via ONp aminolysis. CD44 expression level in several cell types was evaluated using confocal microscopy. The binding and internalization of HA_{34}-containing copolymers by the CD44-positive and CD44-negative cells was determined by means of flow cytometry.

Results and Discussion: The binding affinity of the targeted P-HA_{34}-FITC conjugate to CD44-overexpressing cells *SK-OV-3* and *B16-F10*, was significantly higher relative to the control, non-targeted copolymer (P-FITC), corresponding to ~4-fold and ~17 fold, respectively. Confocal imaging revealed a higher degree of internalization of the HA_{34}-bearing copolymers by the CD44-positive cells than that of P-FITC copolymer. These results indicate that HA_{34} is a suitable ligand for targeting CD44-overexpressing cells. The sensitivity of CD44-positive and CD44-negative cells to the HPMA-copolymers containing both HA_{34} and the anticancer drug Paclitaxel (P-HA_{34}-TXL) is currently being investigated.

Keywords: Hyaluronic Acid (HA), CD44, HPMA.

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HYALURONAN COATED NANOPARTICLES: DOES SIZE MATTER?

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Hyaluronan (HA), a naturally occurring glycosaminoglycan, exerts different biological functions depending on its molecular weight ranging from 4000-10M Da. We and others have demonstrated that high molecular weight (Mw) HA can be covalently attached to the surface of lipid nanoparticles and efficiently target epithelial cancer cells and leukocytes expressing HA receptors. The HA coating endow the carriers with long circulation and active targeting towards HA- receptors (CD44 and CD168) highly expressed on solid tumors and blood cancers. Recent studies have shown that high Mw HA (700K-3M Da) suppresses angiogenesis and immune responses while very low Mw HA (<10KDa), stimulates angiogenesis, suppresses apoptosis and induces several inflammatory cytokines. However, not all literature reports are consistent, for example administration of hyaluronan oligosaccharides to tumor xenografts of various types inhibits rather than stimulates tumor growth and overexpression of hyaluronidase suppresses colon and breast carcinoma growth in xenografts. Therefore, the results vary depending on the system tested. In addition, it is known that the extracellular matrix component HA, which exists physiologically as a high Mw polymer, is cleaved at sites of inflammation where it activates macrophages and epithelial cells. This also suggests that HA obtains new activities and functions after depolymerization. In order to determine the effects of HA on cancer and inflammatory process, we have prepared a set of nanocarriers distinguished by the length of their surface-anchored HA ranging from 6.4kDa to 1.5MDa. The effect of HA Mw on particle size, surface charge and ultrastructure have been determined. While no significant difference in particle size and globular shape was detected, differences in particle surface charge have been observed. In addition, the effect of the HA coated nanoparticles on macrophages, T cells and ovarian cancer cells were tested *in vitro*. No apparent cytotoxic effect or induction of proliferation has been observed on the tested cell lines.

We plan to further use these sets of nanocarriers to test part of the immune response such as lymphocytes activation, cytokine induction, complement activation and interferon responses. In addition we plan to characterize the interaction of HA-coated nanoparticles with their receptors. Understanding the interactions between HA size and charge will help us to tailor the appropriate vehicle for a particular disease and ultimately might aid in establishing a personalized medicine approach using specific drugs in a particular nanocarrier.

P-32

GAGOMERS: CD44-TARGETED CARRIERS FOR DOXORUBICIN

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Doxorubicin (DXR) is a front-line well-established drug agent used against a number of solid tumors, leukemia, and lymphoma. As with most chemotherapeutic drugs, in addition to its beneficial therapeutic effects, there are risks of toxicity and it gives rise to unacceptable adverse effects among them, in particular, cardiotoxicity. These limitations, which are aggravated with the increase in dose levels, are a serious impediment to effective therapy, and therefore created the need of a carrier for doxorubicin. Several approaches are clinically approved including a liposomal formulation, named Doxil®, which is approved for several types of cancers, but this formulation has number of drawbacks, among them is the lack of targeting abilities. Therefore, we devised a novel doxorubicin-carrier system. This carrier is made from biomaterials that include phospholipids that do not create a liposomal form and operates in a cluster bomb manner. The new carrier was termed "Gagomers" (GAGs) since its outer shell is composed of the glycosamonoglycan (GAG), hyaluronan (HA). HA is a major component of the extracellular matrix (ECM), and provides GAGs the ability of longer circulation in plasma as well as retard uptake by mononuclear phagocytes.

In addition, it is responsible for the targeting abilities of GAGs due to its interaction with the transmembrane receptor family, CD44. The CD44 receptors are expressed on almost all cell types, but are undergone conformational changes in cancerous cells and in activated leukocytes. Therefore, GAGs have the ability to interact with the CD44 receptors expressed on tumors and deliver the DXR into the tumors in an efficient manner.
Here, we show that CD44 expression of various tumors (including ex-vivo human xenografts, and primary thyroid carcinoma) is high and could be used for targeting by GAGs. In addition, in highly resistant tumors that efflux drugs utilizing the ABC transporters superfamily, which also correlate with high CD44 expression, DXR could be delivered in a highly efficient manner.

Our hypothesis is that GAGs operate in these highly resistant tumors by diverting the ratio of influx/efflux of the DXR into the cell in favor of the influx procedure, and thus retain in the tumor cell and induce cell death.

P-33

SURFACE ENGINEERED POROUS SILICON-BASED NANOSTRUCTURES FOR CANCER THERAPY

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In recent years, mesoporous Silicon (PSi) has emerged as a promising and versatile biomaterial for drug delivery applications owing to its biodegradability and biocompatibility. PSi exhibits significant advantages compared to synthesized mesoporous materials when drug delivery vehicles are considered. The fabrication of PSi is a simple procedure, where the porosity and pore size can easily be tuned by anodization parameters. In addition, the surface chemistry of PSi can easily be modified to produce surfaces favorable of drug adsorption and entrapment.
Our work aims at developing new PSi-based nanostructures for delivering antineoplastic drugs. We focus on engineering the PSi nanostructure and surface chemistry to control the loading and release of Mitoxantrone (MTX, a model anticancer drug). Different PSi films are prepared by electrochemical anodization of Si in hydrofluoric acid solutions. Etching conditions are adjusted to maximize the drug loading within the porous nanostructure. We find that PSi films characterized by interconnecting cylindrical pores ranging in diameter from 5-10 nm, average thickness of 2.3µm, and porosity of 64% are optimal for loading MTX. Following the electrochemical etch step, the films are chemically modified by thermal hydrosilylation (with 1-dodecene or undecylenic acid), and the drug payload is incorporated within the porous nanostructure. Two loading routes are explored: (i) physical adsorption, and (ii) covalent attachment of the drug. Significant differences in drug release profiles are found between the dodecene-modified PSi and freshly-etched samples. Thus, by changing the surface properties of the PSi from moderately hydrophilic to hydrophobic, the release of MTX can be slowed by a factor of 20. The undecylenic acid-modified PSi samples exhibit different drug release rates due to the conjugation of the drug to the carboxylic acid-functionalized PSi via Si-C bonding. The release of the drug is characterized by a two-step mechanism including oxidation followed by dissolution of the PSi matrix. Cellular assays confirmed the cytotoxicity of the MTX released from the different PSi-loaded systems towards MDA-MB231 cancer cell lines.

Keywords: Porous Si, drug loading, controlled drug release, cancer therapy, nanotechnology

P-34

BIOACTIVE CERAMIC SCAFFOLD CONJUGATED PLGA PARTICLES FOR BONE TISSUE ENGINEERING

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Introduction

Every year about one million cases of skeletal defects require bone graft procedures. Tissue engineering (TE) is an alternative approach for treatment which eliminates problems of donor site scarcity, immune rejection and pathogen transfer. Composite scaffolds for bone tissue engineering made of both ceramic and polymeric components have the advantage of being osteocunductive, while enabling a controlled release rate of growth factors, such as bone morphogenetic protein-2 (BMP2). BMP2 is a regulatory molecule related to the TGF-β growth factor family, which is involved in skeletal tissue formation and is known to be very important in regulation of proliferation and osteogenic differentiation of mesenchymal stem cells (MSC). We propose a ZrO_2 ceramic scaffold conjugated to a controlled delivery system of PLGA (poly-lactic-co-glycolic-acid) microspheres loaded with BMP2, as a bioactive scaffold supporting bone tissue regeneration.

Materials and Methods

PLGA microspheres were prepared by the oil-water-oil solvent-extraction technique. The particles were attached to the ceramic matrices using different approaches. Microspheres were characterized by scanning electron microscopy (SEM) and a coulter counter. Release kinetics and loading capacity of BMP2 were studied by Elisa and microBCA assays. MSC were seeded on the scaffold and their attachment and proliferation were studied.

Results

SEM imaging of the PLGA particles embedded in the ZrO_2 scaffold showed that particles posses a spherical structure. A slower release rate of BMP2 was observed from the embedded microspheres, which could result from adsorption of the BMP2 to the scaffold. hMSCs were seeded on ZrO2 scaffolds and growth of the cells was measured over 60 days using the AlamarBlue cell viability assay. Growth was not affected by seeding density and the hMSCs grew extensively and filled the scaffold pores.

Discussion and Conclusions

A continuous release of BMP2 from the embedded PLGA microspheres and the successful cultivation of hMSCs suggest that the composite scaffold could give a favored surrounding for bone TE. More research is currently done in our lab on the basis of these promising results.

Acknowledgments:
We would like to thank the Niedersachsen foundation for supporting this research.

Keywords:
Tissue engineering, PLGA, scaffolds.

P-35

PORCINE ARTERIAL EXTRACELLULAR MATRIX BIOGRAFT - A POTENT REPLACEMENT FOR SMALL CALIBER BLOOD VESSELS

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Introductions

Despite a clear clinical need for a functional arterial graft, success has been limited to arterial replacements of large-caliber vessels. In order to assess the efficacy of porcine arterial extracellular matrix (ECM) to serve as small caliber allograft, we performed a preclinical trial study in porcine. The purpose of this preclinical trial was to evaluate the ECM biograft in terms of patency, thrombogenicity, immunogenicity and the ability to withstand the blood pressure.

Materials & Methods

The preclinical trial was performed in six porcine and includes two arms: decellularized and recllellulrized ECM allografts. The recellularized allograft lumen was reseeded with autologous saphenous vein endothelial cells. The reseeded cells were grown under a pulsatile flow in an arterial bioreactor system for 21 days. The allografts were anastomosed in an end-to-end to the common carotid artery. Each animal underwent two vascular procedures, the allograft was anastomosed to the right carotid and sham operation was performed at the left carotid as an internal control. The carotid artery blood flow rate was measured by an ultrasound flow-meter. The allograft patency was evaluated with X-ray angiography every week. The allograft explants were subjected to pathologic evaluation by masson's trichrome and verhoeff staining, and to CD-31, laminin and α-SMC immunohistological staining.

Results

The allografts were continually stitched with prolene® (6-0) and then blood flow was renewed. No hemorrhage or rupture was observed. Moreover, a normal blood pulsate flows were measured at the transplanted allografts. Furthermore, the blood flow-rate in the transplanted allograft resembled a normal flow- rate with an average of 145 ml/min, as was measured by the ultrasound flow-meter. X-ray angiography images revealed that decelularized-ECM allograft patency remained for 4 weeks, while the recellularized-ECM allograft patency remained for the full period of the preclinical trial (6 weeks). Pathological findings revealed a minor immunogenic response at the adventitia layer that was characterized by macrophages infiltration, in both preclinical arms, which was comparable to the sham operation. The histological and immunohistological stains of the recellularized biografts revealed that their tunica media was refilled with smooth muscle cells. Their tunica intima was coated with endothelial cells producing the laminin protein that embedded within basement membrane. However, in the decellularized allografts stenosis was observed which occurred as a result of intimal fibrobalast hyperplasia. These preclinical findings indicate that porcine ECM recellularized with endothelial cells has a great potential to serve as a future biograft for arterial tissue engineering.

Acknowledgments: This work was supported by the NOFAR grant from the Chief Scientist at Israel. The authors wish to thank Proffesor Aharon Hoffman and Dr. Tony Karram for the vascular operations.

Keywords: arterial biograft, extracellular matrix, anastomosis.

*This abstract describes a student's research work.

P-36

NATURAL EXTRACELLULAR MATRIX PLATFORMS TOWARDS ENGINEERING OF THICK CARDIAC-LIKE TISSUE CONSTRUCTS

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Introduction: We have previously reported the successful isolation of thin porcine cardiac ECM (pcECM) slices which manifested bio-mechanical properties relevant for myocardial tissue engineering [1]. As cardiac tissue could reach a thickness of 12-15 mm, the development of thin constructs offers limited regeneration capacity. Currently, the achievement of thick myocardial-like tissue constructs is limited due to diffusion limitations (~100 μm), and the lack of proper vascular network enabling the delivery of nutrients and oxygen [2]. Thus the development of a support system that would enable the cultivation of thicker constructs is required. The present work focuses on the optimization of the decellularization procedure for thicker tissue constructs and the development of a novel supportive bioreactor system.

Results: Our previousely reported decellularization procedure [1] was optimized to obtain thick pcECM (10-15 mm) by increasing trypsin activity and the introduction of sonication and/or perfusion through built-in vasculature. The Increase of trypsin activity as well as the use of sonication and/or perfusion enabled a better decellularization procedure compared to control. No cellular remains (Red for cytoplasm and black for nuclei) were observed with Masson trichrome staining of histological cross sections. Oil-red staining showed remaining of adipocytes. SEM and multiphoton microscopy showed preserved structural characteristics, supportive of cellular growth. Realtime RT-PCR analysis of the TNFα/GAPDH expression ratio in bone marrow derived macrophages (BMM), revealed low stimulation of pcECM exposed cells, compared to native cardiac tissue. Vascular network functionality was preserved to the first three-four branches from the main coronary vessels (assessed by corrosion casting and perfusion of fluorescently labeled dextran). A novel supportive bioreactor system is currently being developed to enable perfusion.

Conclusions and significance: We have successfully isolated thick pcECM which is non immunogenic and preserves ultrastructural properties as well as inherent vascular network. A novel bioreactor system is currently being developed that would enable the control of pulsate flow, electrical and mechanical pre-conditioning. Future work will focus on the evaluation of different cell population seeding, cultivation under static conditions, and the optimization of dynamic cultivation parameters. This system could potentially allow future studies on the delivery and controlled release of therapeutics in a cardiac mimetic environment *in-vitro*.

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Keywords: Myocardial tissue engineering, Bioreactors, Extracellular matrix (ECM), porcine heart

P-37

INTEGRATION OF MULTIPLE CELL-MATRIX INTERACTIONS INTO INERT ALGINATE SCAFFOLDS FOR CARDIAC TISSUE REGENERATION

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Background and aims: Engineering a functional cardiac tissue *in vitro* is one of the most challenging tasks for tissue engineers. In this research, we aimed to reconstruct the microenvironment promoting cardiac tissue regeneration by presenting multiple cell-matrix interactions, in a similar manner to their presentation by the extra-cellular matrix (ECM) *in vivo*. Thus, two fibronectin-derived peptides, (RGD and heparin binding peptide (HBP), were bound to alginate scaffold, mimicking the specific interactions of ECM with integrin and syndecan on cell membrane, respectively

Methods and Results: The peptides (GGGGRGDY, GGGGSPPRRARVTY or their combination) were covalently-attached to alginate via the carbodiimide chemistry, creating an amide bond between the peptide terminal amine group and the alginate carboxylic group. High efficiency of peptide attachment and uniform distribution in the scaffold were confirmed by using fluorescently-tagged peptides. Peptide binding did not have an effect on scaffold internal morphology (e.g., porosity by Scanning Electron Microscope) or matrix stiffness. The HBP/RGD-modified scaffold was more favorable compared to that with single peptide- or unmodified alginate scaffolds, as reflected by the increased AKT phosphorylation, indicating to the activation of adhesion-dependant pathway and pro-survival signaling. Furthermore, already by day 7 in culture, the cardiomyocytes in HBP/RGD scaffold reorganized their myofibrils and revealed extensive striation, while the non-myocyte cells constructed the cardiomyocyte-supporting sheets. A similar morphology was obtained in the RGD-modified scaffolds only on day 14. In contrast, the HBP-modified and unmodified scaffolds had no such as effect on cardiac reorganization. Finally, Connexin-43 and N-Cadherin expression profiles presented better tissue maturation and regeneration of a functional cardiac muscle tissue within the scaffolds with the multiple functional cues.

Conclusions: Our data establish the potential use of HBP/RGD alginate scaffolds as a better ECM-mimicking microenvironment for inducing regeneration of functional cardiac tissue, *in vitro.*

Acknowledgement: We thank Olga Kryukov for excellent technical assistance in confocal microscopy. Prof. Cohen holds the Claire and Harold Oshry Professor Chair in Biotechnology.

Keywords: RGD, heparin binding peptide, alginate scaffold, cardiac tissue regeneration.

*The results presented in the abstract are part of a PhD thesis.

P-38

TGFβ1/AFFINITY-BOUND ALGINATE SCAFFOLD ENHANCES CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS (HMSC)

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Background and aims: Mesenchymal stem cell differentiation profile depends on the environment wherein cells reside, especially on the spatio-temporal presentation of differentiation-inductive growth factors. Herein, we aimed to reconstruct the microenvironment, which promotes chondrogenic differentiation, by presenting the chondro-inductive Transforming Growth Factor-b1 (TGFb1), in a similar manner to its presentation by the extra-cellular matrix. Thus, TGFb1 was affinity-bound to alginate-sulfate containing scaffold, mimicking the specific interactions of this factor with heparan sulfate.

Methods and results: TGFb1/affinity-bound to alginate-sulfate containing scaffolds were prepared by a freeze-dry technique (200ng protein/scaffold). *In vitro* release study, as shown by ELISA, showed the sustained release of TGFb1 for 7 days from the affinity-binding alginate scaffolds, compared to the burst release of nearly 100% of the entrapped TGFb1 from alginate scaffolds (p, interaction<0.0001, 2-way ANOVA). TGFb1 retained its biological activity, as assessed by its ability to enhance collagen deposition in fibroblast culture. HMSC were seeded into TGFb1/affinity-bound scaffolds (at a cell density of 300,000 cells/scaffold), and TGFb1-induced chondrogenic signal-transduction pathways were tested by Western Blot. Prolonged expression of phosphorylated Smad2 and increased phosphorylation level of ERK1/2 for up to 14 days in hMSCs culture within TGFb1/affinity-binding scaffolds, indicate the long-term activity of TGFb1 in this system. Masson's trichrome staining of the 14 days-old cell constructs demonstrated massive deposition of collagenous material within the TGFb1/ affinity-bound scaffolds. Specifically, the production/secretion of collagen type II was more pronounced and the cells had the round morphology of committed chondrocytes in these scaffolds.

Conclusions: These data indicate the potential use of the affinity-binding alginate scaffolds combined with spatial presentation of TGFb1 for reconstruction of the microenvironment for neo-cartilage formation.

Acknowledgement: This work is supported by the Azrieli Foundation (TR) and Israel Science Foundation (ISF). Prof. Cohen holds the Claire and Harold Oshry Professor Chair in Biotechnology.

Keywords: affinity binding, alginate scaffold, Transforming Growth Factor b1, Human mesenchymal stem cells, chondrogenesis

*The results presented in the abstract are part of a PhD thesis.

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MANIPULATION OF HEMATOPOIETIC STEM CELLS USING TARGETED NANOPARTICLES ENTRAPPING RNAI

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Hematopoietic stem cells (HSCs) are the most investigated stem cells. HSCs are rare cells, only 1 of 10^5 bone marrow (BM) cells is considered as a HSC. HSCs sit atop a hierarchy of progenitors that can progressively differentiate to several lineages and give rise to over a billion new mature blood cells including red blood cells, myeloid and lymphoid cells every day. HSCs are mostly resided in the BM in a quiescent stage. When they enter the cell cycle, they have a unique ability of self-renewal, by dividing asymmetrically producing an additional HSC and a progenitor cell. HSCs can be identified and isolated by monoclonal antibodies directed to surface markers such as stem cell antigen -1 (Sca-1) and c-kit (CD117). HSCs depend on their microenvironment, the bone marrow niche, for regulation of quiescent, self-renewal and differentiation. As a result of the essential interaction with the niche, HSCs have to be investigated *in vivo* and this raise the need to develop a delivery system to target HSCs in BM environment.

RNA interference (RNAi) is a ubiquitous and highly specific mechanism of gene silencing. RNAi has emerged as a powerful tool for elucidating gene function and identifying potential drug targets. RNAi can also be exogenously activated by introducing short double-stranded RNAs (siRNAs) or MicroRNAs (miRNAs) into the cytoplasm of cells. To realize the potential of RNAi for in vivo target discovery and therapy there is a need to overcome the considerable hurdle of intracellular delivery across the plasma membrane into specific cells. RNAi are not taken up into most cells in the absence of a transfection reagent. For many cells, mixing RNAi at nanomolar concentrations with a lipid transfection reagent can efficiently induce gene silencing. However some cells such as primary lymphocytes and HSCs remain highly resistant to lipid transfection. Systemic delivery to HSCs remains a significant barrier for exploring the full potential of RNAi in this context.

Our hypothesis is that targeted nanoparticles entrapping RNAi can be developed to induce *in vitro* and *in vivo* silencing in HSCs and early progenitors. This delivery system contains lipid-based nanoparticles (NPs) covalently attaching a monoclonal antibody against a specific HSCs and early progenitors' cell surface marker (Sca-1). The NPs can entrap different RNAi targeting a variety of genes. Using this strategy we plan to identify key genes responsible for HSCs self-renewal properties. It will provide a powerful technique to investigate the contribution of individual genes in maintaining the phenotypic and functional properties of HSCs, and ultimately may provide a way to improve engraftment during transplantation.

P-40

MANIPULATING HAIR GROWTH BY RNAI TARGETING TO HAIR FOLLICLE BULGE STEM CELLS

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Adult tissue-specific stem cells (SC) have the capacity to self-renew and generate functional differentiated cells that replenish lost cells throughout an organism's lifetime. SC behavior, and in particular the balance between self-renewal and differentiation, is ultimately controlled by the integration of intrinsic factors with extrinsic cues supplied by the surrounding microenvironment in the form of secreted and cell surface molecules. This valuable microenvironment is known as the SC niche and represents a defined anatomical compartment.

The hair follicle (HF) corresponds to miniature organ, which during the normal lifespan of a human regenerates itself more than 10 times. Existing follicles undergo cycles of growth (anagen), regression (catagen) and rest (telogen) where the responsible cells for this remarkable process are the HF SCs (HFSCs) which reside in the bulge region. The hair cycle represents a remarkable model for study the regulation of SC quiescence and activation in the context of SC niche cross-talk. In recent years, the advances in SCs isolation methods enable the existence of SCs transcription profile, revealing a list of genes express highly in HFSCs.

In-vivo manipulations of gene expression levels in SCs at their niche may clarify gene function and uncover the major players that are involved in regulation of SCs fate. However, elucidating gene function using classical genetic methods, such as transgenic mice, consume major resources and time for each candidate gene examined. In addition, gene knockout harbor a complete ablation that harden results interpretations by promoting compensation mechanisms.

In this work, we aim to develop topical RNAi delivery platform to target specifically the HFSCs and to exploit this technology as a new approach to study SC biology in general and more specifically to study how to manipulate HFSCs fate.

Keywords: Hair follicle, RNAi, siRNA, delivery, stem cells

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MICRONEEDLE-INDUCED CHANGES IN THE HUMAN SKIN BARRIER: QUANTIFICATION BY TRANSEPIDERMAL WATER LOSS (TEWL)

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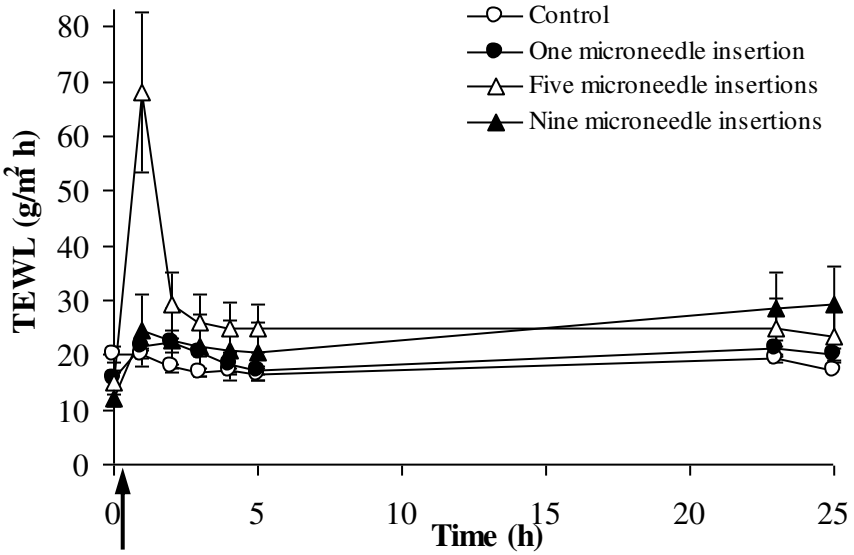
Microneedle arrays have aroused great interest in recent years for their potential to promote transdermal drug delivery. Yet there are still questions on how parameters such as needle dimensions or repeated insertions perturb skin barrier function. Our aim was to analyse this by using TEWL as a rapid screening system[1].

To this end, dermatomed Caucasian human skins (NDRI, Philadelphia PA), of 330µm-thickness, were inserted in Franz cells. Basal TEWL was measured with a calibrated and dedicated AquaFlux meter[2] (Biox Systems, London UK) that slotted in the donor chamber. Each skin was detached from the cell and a specific array of conical polymeric microneedles was manually applied. Tested variables were needle length, needle interspacing distance, insertion time and multiple insertions. Each skin was then re-mounted in the cell and TEWL measured over a further 25 h.

Sample Results: Effect of multiple array insertions ($n \geq 3$).



Insertions caused an immediate TEWL increase followed by a gradual fall to near baseline values. This suggests the barrier is being perturbed by the newly-created pores but then partially recovers as these contract under elastic forces. Importantly, 9 insertions yielded a smaller TEWL peak than 5 insertions, probably because the skin is so compressed that the barrier is actually augmented[3]. For 9 insertions, gradual tissue decompression explains the observed slow rise in TEWL over 5 to 25h. Taken together, all the results showed that TEWL can be used to rapidly quantify microneedle-induced skin poration in real time.

Acknowledgements: We are grateful to Ryan Donnelly's team (Queens University, Belfast, UK) for fabricating the microneedle arrays.

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P-42

RAPAMYCIN CRYSTALLIZATION AND RELEASE FROM STENT

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Drug eluting stents have been used for the reduction of restenosis after stent insertion. The drug, rapamycin or paclitaxel, is loaded in a polymer carrier coating onto stents. Rapamycin is a macrolide that was originally used to prevent rejection of organs.

Rapamycin surface crystallization process consists of two steps, seeding and crystallization (Scheme 1). The study shows that the surface crystallization is a function of two main parameters at constant temperature, concentration of drug at the crystalline solution and the time of incubation at crystalline solution.

In vitro results show that stents released the drug in a controlled manner for weeks. The stents released 0.75% to 2.2% of the total amount of drug-stent loaded after 10 Days incubation in a phosphate Buffer solutions pH 7.4, 100 rpm.

Figure 1

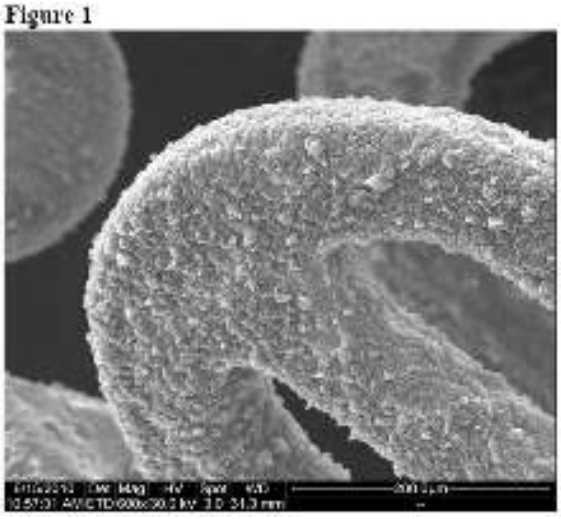
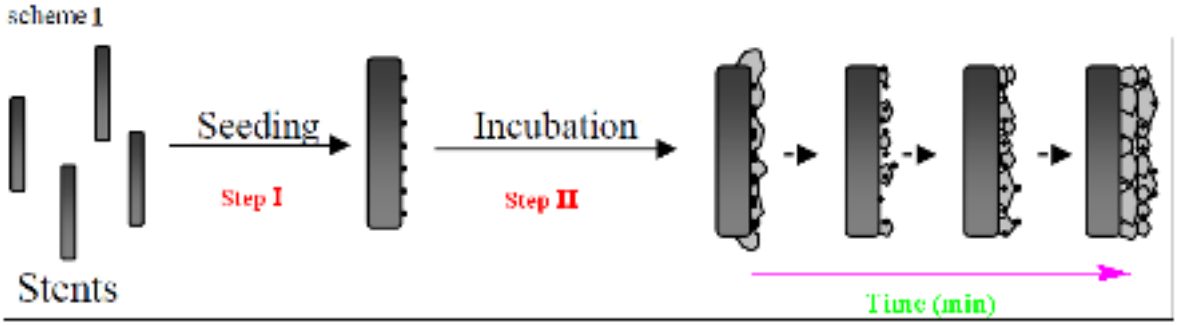


Figure 2

scheme 1



P-43

EFFECT OF ULTRASOUND AND CHEMICAL PENETRATION ENHANCERS ON TRANSPORT PHENOMEMNA OF THE CHORIOAMNION MEMBRANE

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In recent years, many studies in biomedicine have been devoted to finding alternatives to invasive medical procedures. Among these is the use of chemical penetrating enhancers (CPEs) and ultrasound for transdermal drug delivery (TDD) and sensing. In these applications, a CPE and/or ultrasound are applied on a biological membrane in order to enhance its transport phenomena. The proposed mechanisms of action are based on the ability of CPEs to affect the skin by altering its lipid and protein structures, thus affecting partitioning behavior and/or the diffusion coefficient. The ultrasound enhanced permeability is attributed to the cavitational effects induced by low frequency ultrasound. The aim of this research is to evaluate the effect of CPEs and their combination with ultrasound on the chorioamnion membrane transport phenomena. Because invasive prenatal diagnostic methods in practice today (amniocentesis and chorionic villus sampling) can induce miscarriage (up to 2%), methods enabling non-invasive sampling of ammonic fluid to detect genetic abnormalities and delivery of drugs directly to the fetus will be beneficial.

In this study, *in vitro* experiments were performed on post-delivery human chorioamnion membranes (authorized by the Helsinki- committee of Hillel Yaffe Medical Center, Israel) to evaluate the enhancing effect and mechanism of different groups of CPEs (i.e. fatty acids, alcohols and surfactants) and/or ultrasound on the chorioamnion membrane transport phenomena.

The permeability experiments demonstrated that the chorioamnion membrane's permeability varies for different donors and membrane sites tested for the same donor. Most CPEs gave a relatively moderate enhancement. Nevertheless, Sodium Lauryl Sulfate (SLS) and limonene with amino-amides, such as bupivacaine raised permeability by 6-fold, while their combined application with ultrasound enhanced the permeability by 28-fold. We propose that the increased permeability in the human chorioamnion membrane is related to the partitioning coefficient of octanol/water of the different CPEs. Moreover, we hypothesized that for chorioamnion membranes exposed to ultrasound the penetration of the CPEs into the membrane is deeper and faster. This hypothesis will be further investigated in the research.

Keywords: chemical penetration enhancers, ultrasound, transport phenomena and Chorioamnion membranes.

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ULTRASOUND TRIGGERED POLYMER DEGRADATION, AN APPROACH FOR ULTRASOUND TRIGGERED INFERIOR VENA CAVA FILTER RESORPTION

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Venous Thromboembolism is a pathological condition when micro- thrombi formed in the normal fibrinolytic system in the blood stream grow and form blood clots within a vein. The blood clot known as thrombosis detaches from the vessel wall and travels in the blood stream, risking in a block of the blood stream in small blood vessels. Today existing medical treatments include: anticoagulation medication, tissue plasminogen activator which catalyzes blood clot breakdown, and Inferior Vena Cava (IVC) Filter which is a medical metal device inserted by catheter to the vena cava physically preventing the passage of large life-threatening blood clots in the blood stream. Popular complications in use of the filter are: malposition of the filter at time of placement, migration of the filter due to dynamic flow and the most dangerous complication is when large or many thromboses clog the filter resulting in vein block. Due to the popular complications a new demand has arised: a filter which can be opened without need of surgical intervention when the filter is not necessary or is at high risk of clogging.

The research objective is to find an optimal model for an improved IVC filter device according to the new demand. We propose an improved filter in which metal wires will be connected by a degradable polymer joint. The polymer properties enable enhanced degradation on demand by use of focused ultrasound (FUS) radiation as an energy source. Upon degradation, the metal wires polymer joint will open, enabling free flow of the blood stream, while the metal wires retract and stay attached to the blood vessel wall.

In this study, the desired properties of an optimal model considering the filter device properties and human body physiological conditions have been examined. Finding both a suitable material and an optimal FUS method are the targets. The first and intuitive material examined was PLGA polymer at different ratios due to its suitable properties. It is biodegradable, biocompatible, bio stable and already FDA-approved for therapeutic applications. At first the polymer strength and ability to connect metal wire were examined. The second step was determining the polymer properties affected by FUS radiation. After FUS radiation of different lactic to glycolic ratios in the copolymer it was found that PLGA 75:25 is most sensitive to FUS resulting in higher degradation rate. The FUS increases PLGA rate of degradation due to both cavitation and thermal effect. Most effecting parameters of FUS radiation are the intensity of the wave and radiation time. The higher the intensity and the longer the radiation result in higher degradation rate.

In future studies we'll examine ultrasound triggered degradation and opening of the filter in flow conditions mimicking blood flow in vena cava.

Keywords: ICV filter, PLGA, Ultrasound (US), Focused ultrasound (FUS), degradation rate.

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NONDESTRUCTIVE DETERMINATION OF DRUG-TO-LIPID RATIO OF LIPOSOMAL FORMULATION

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A novel method for monitoring the drug-to-lipid ratio for optimization of quality of liposomal formulation of doxorubicin (Doxil) is described. The method is based on precise measurements of ultrasonic properties of the liposome suspension: sound velocity, absorption of ultrasound, acoustic impedance and their temperature dependence, which are sensitive to composition of liposomal formulations. By measuring at least five independent ultrasonic parameters, one can obtain a single-valued solution of five equations and determine all main components of the PEGylated liposomal formulation: buffer solution, salt (ammonium sulphate), lipid mixture (HSPC+PEG-DSPC), cholesterol and doxorubicin:

$$(SV)^T = k_1(\%\text{Buf}) + k_2(\%\text{Salt}) + k_3(\%\text{Lipid}) + k_4(\%\text{Chol}) + k_5(\%\text{DOX})$$
$$d(SV)/dT = k_6(\%\text{Buf}) + k_7(\%\text{Salt}) + k_8(\%\text{Lipid}) + k_9(\%\text{Chol}) + k_{10}(\%\text{DOX})$$
$$(UA)^T = k_{11}(\%\text{Buf}) + k_{12}(\%\text{Salt}) + k_{13}(\%\text{Lipid}) + k_{14}(\%\text{Chol}) + k_{15}(\%\text{DOX})$$
$$d(UA)/dT = k_{16}(\%\text{Buf}) + k_{17}(\%\text{Salt}) + k_{18}(\%\text{Lipid}) + k_{19}(\%\text{Chol}) + k_{20}(\%\text{DOX})$$
$$(AI)^T = k_{21}(\%\text{Buf}) + k_{22}(\%\text{Salt}) + k_{23}(\%\text{Lipid}) + k_{24}(\%\text{Chol}) + k_{25}(\%\text{DOX})$$

where $(SV)^T$, $(UA)^T$, $(AI)^T$ are, respectively, the sound velocity, ultrasound absorption and acoustic impedance at a fixed temperature T, $d(SV)/dT$ and $d(UA)/dT$ are sound velocity and ultrasound absorption temperature dependences, and constants k_1 through k_{25} are derived by correlation with analyses performed by liquid chromatography.

Comparative analysis of the ultrasonic method with a standard liquid chromatography method have produced linear calibration curves for doxorubicin and lipids with correlation coefficients higher than 0.95. The range for doxorubicin, lipids and cholesterol evaluation by the Ultrasonic Analyzer of NDT Instruments Ltd. (Jerusalem), based on standing cylindrical ultrasonic waves of 10 MHz, is 0.05 to 50.0 mg/ml. It is thus possible to monitor all main components of the liposome formulation directly and to compare the therapeutic efficacy of various drug-to-lipid ratios. Advantages of the proposed method include its nondestructive nature, no need for sample pre-treatment, ease-of-use, and rapid results.

Keywords: Ultrasonic standing wave resonator, Nondestructive testing, Doxil

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MORPHOLOGICALLY DIRECTED RAMAN SPECTROSCOPY AS A NOVEL TOOL TO AID DEVELOPMENT OF DRUG DELIVERY SYSTEMS

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One of the fundamental steps in the formulation of a drug delivery system is the bringing together of several individually manufactured ingredients which are then processed to achieve the final dosage form. Up until the formulation process, it is relatively straightforward to measure critical quality attributes such as particle size, shape and chemical identity of the input materials using well established techniques such as laser light scattering, particle image analysis and traditional spectroscopic methods. A much more difficult challenge is to be able to characterise the size, shape and chemical identity of the individual ingredients once they have been through this formulation process. This may be especially important when trying to understand any possible impact of the formulation process on the size, shape and chemical distribution of the API since this may have important implications for the bioavailability or bioequivalence of the drug. The technique of NIR chemical imaging has been established as a useful technique to measure the homogeneity of powder blends for tabletting applications [1], however technical limitations impact its applicability for smaller particle sizes found in more complex drug delivery systems.

In this presentation we describe a novel approach using a hybrid technique of Morphologically directed Raman Spectroscopy to measure the size, shape and chemical identity of particles in formulated drug delivery systems. Initially high quality microscopic images of individual particles are captured allowing characterisation of their size and shape. Selected particles are then targeted in an automated manner with a Raman microprobe to obtain a chemical ID for each individual particle. Particle size and shape distributions can then be constructed for each of the ingredients and compared directly with measurements made before the formulation process. Results for a nasal spray formulation are presented and implications for a number of different types of pharmaceutical formulations are discussed.

[1] Ma H, Anderson CA, Characterization of pharmaceutical powder blends by NIR chemical imaging. Journal of Pharmaceutical Sciences 97:3305-3320

Keywords: Raman, Imaging, Size, Shape, API

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