Third International Nanomedicine and Drug Delivery Symposium

September 26-27, 2005

Venue:
Holiday Inn, Baltimore Inner Harbor
Baltimore, Maryland, USA

Co-organizers

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Alexander (Sasha) Kabanov (*Univ. Nebraska Medical Center, Omaha*)

Kalle Levon (*Polytechnic Univ., New York*)

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Preface

The organizing committee would like to welcome you to the Third International Nanomedicine and Drug Delivery Symposium, 2005 in Baltimore, Maryland.

The convergence of recent advances in nanotechnology with modern biology and medicine has created the new research domain of nanobiotechnology. The use of nanobiotechnology in medicine is termed nanomedicine.

Nanomedicine research includes the development of diagnostics for rapid monitoring, targeted cancer therapies, localized drug delivery, improved cell material interactions, scaffolds for tissue engineering, and gene delivery systems.

The focus of this symposium will be on recent advances in nanomedicine with emphasis on the delivery of bioactive agents for therapeutic and diagnostic purposes using polymeric biomaterials. We hope you enjoy the symposium.

Contributors and Sponsors

Major support provided by
grants from the National Institute of Biomedical Imaging and Bioengineering and
National Cancer Institute (1R13 EB005534-01)

Support also provided by:

Index

	Page
Program.....	6
Invited speakers' abstracts and biosketches.....	20-64
Abstracts of poster presentations.....	65-106
Author index.....	107-109

Program

Monday September 26, 2005

Page

8:00-9:00 AM
Registration / Continental Breakfast / Poster Mounting (8:00-8:45 AM)

9:00-9:10 AM
Introductory Remarks
David Knapp, PhD
Dean, University of Maryland School of Pharmacy

Natalie Eddington, PhD
Chair, Dept. of Pharmaceutical Sciences, University of Maryland, Baltimore

Hamid Ghandehari, PhD
Associate Professor and Director, University of Maryland Center for Nanomedicine and Cellular Delivery

9:10-9:40 AM
Keynote Presentation
Non-viral gene delivery: basic science or clinical reality? 21
Francis Szoka, Jr., PhD
Professor, Biopharmaceutical Sciences and Pharmaceutical Chemistry, University of California San Francisco

Moderator:
Alexander (Sasha) Kabanov, PhD
Parke-Davis Professor of Pharmaceutical Sciences and Director, Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center

9:40-10:40 AM
<u>Session I:</u> Nanobiomaterials: engineering and characterization

Moderators:
Kam Leong, PhD
Professor, Department of Biomedical Engineering, Johns Hopkins University

William Bentley, PhD
Professor and Director of Bioengineering Program, University of Maryland, College Park

Surface characterization of nanosystems 23
Martyn Davies, PhD
Professor of Biomedical Surface Chemistry, School of Pharmacy, University of Nottingham

Recombinant polymers as platforms for nanoconstructs 25
Kristi Kiick, PhD
Assistant Professor, Department of Materials Science and Engineering, University of Delaware

10:40-11:00 AM
Coffee break / Poster viewing

11:00 AM-12:00 PM
Session II: Subcellular fate and function of nanoconstructs

Moderators:
Peter Swaan, PhD
Associate Professor and Co-Director, University of Maryland Center for Nanomedicine and Cellular Delivery

Justin Hanes, PhD
Associate Professor, Department of Chemical and Biomolecular Engineering, Johns Hopkins University

Drug delivery systems for remediation of cellular hypoxic damage 27
Tamara Minko, PhD
Associate Professor, Department of Pharmaceutics, Rutgers, The State University of New Jersey

Nanosystems biology: study of cellular processes in live single cells 29
James Heath, PhD
Elizabeth W. Gilloon Professor of Chemistry, Division of Chemistry and Chemical Engineering, California Institute of Technology

12:00-1:15 PM
Lunch / Poster and Exhibit Viewing

1:15-3:15 PM
<u>Session III:</u> Drug and gene delivery

Moderators:
Sonke Svenson, PhD
Senior Research and Development Scientist, Dendritic NanoTechnologies Inc.

Stephen Hoag, PhD
Associate Professor of Pharmaceutical Sciences, University of Maryland, Baltimore

Smart delivery systems for biomolecular therapeutics 31
Patrick Stayton, PhD
Professor, Department of Bioengineering, University of Washington

Ligand-targeted nanoparticles for siRNA delivery 33
Martin Woodle, PhD
Chief Scientific Officer, Intradigm Corporation

Template-synthesized magnetic nanotubes for drug delivery. 35
Sang Bok Lee, PhD
Assistant Professor, Department of Chemistry and Biochemistry, University of Maryland, College Park

Dendrimers – a promising approach to tailored carriers in drug delivery applications 37
Sonke Svenson, PhD
Senior Scientist, Dendritic Nanotechnologies

3:15-3:30 PM
Coffee break / Poster viewing

3:30-5:30 PM
<u>Session IV:</u> Bioimaging, diagnostics, and radiotherapy

Moderators:
William Eckelman, PhD
Molecular Tracer, LLC, Bethesda, MD

Martin Woodle, PhD
Chief Scientific Officer, Intradigm Corporation

Nanomaterials functionalized with oligosaccharide cell surface receptors: new approaches to biosensing, diagnostics and drug delivery. 39
Philip DeShong, PhD
Professor, Department of Chemistry and Biochemistry, University of Maryland, College Park

Polymers and polymerization in molecular imaging 41
Alexei Bogdanov, PhD
Professor, Radiology and Cell Biology, University of Massachusetts Medical School

Targeted delivery of radionuclides to sites of angiogenesis 43
Bruce Line, MD
Director, Division of Nuclear Medicine, and Professor of Diagnostic Radiology, University of Maryland, Baltimore

Multifunctional near-infrared nanoparticulate system for diagnosis and therapy 45
Mostafa Sadoqi, PhD
Assistant Professor, Department of Physics, St John's University

5:30-7:00 PM
Refreshments / Poster viewing ***(5:45-6:45 PM-presenters will be available by posters)***

Tuesday September 27, 2005

Page

8:00-9:00 AM
Continental breakfast / Poster viewing

9:00-10:30 AM
Session V: Targeted delivery of anticancer agents (1)

Moderators:
Edward Sausville, MD, PhD
Associate Director of Clinical Research, Greenebaum Cancer Center, Professor of Medicine, University of Maryland, Baltimore

Tamara Minko, PhD
Associate Professor, Department of Pharmaceutics, Rutgers, The State University of New Jersey

Water-soluble polymers for cancer therapy: from concept to clinic 47
Jindrich Kopecek, PhD
Distinguished Professor, Departments of Pharmaceutics and Pharmaceutical Chemistry, and Bioengineering, University of Utah

Nano-scale ligand-targeted drug delivery systems 49
Theresa M. Allen, PhD
Professor, Department of Pharmacology, University of Alberta

Polymer micelles with cross-linked ionic cores for delivery of anticancer agents 51
Tatiana K. Bronich, PhD
Associate Professor, College of Pharmacy, University of Nebraska Medical Center

10:30-10:45 AM
Coffee break / Poster viewing

10:45-11:45 AM
Session VI: Targeted delivery of anticancer agents (2)

Moderators:
Angelika Burger, PhD
Associate Professor, Department of Pharmacology & Experimental Therapeutics, University of Maryland, Baltimore

John P. Fisher, PhD
Assistant Professor, Department of Chemical and Biomolecular Engineering and Bioengineering Graduate Program, University of Maryland, College Park

Thermally responsive polypeptides for targeted delivery of therapeutics to solid tumors 53
Ashutosh Chilkoti, PhD
Associate Professor, Associate Director, Center for Biologically Inspired Materials and Materials Systems, Department of Biomedical Engineering, Duke University

VIP- Receptor targeted phospholipid nanocarriers for anticancer drug delivery 55
Hayat Onyuksel, PhD
Professor of Pharmaceutics and Bioengineering, Assistant Head and Director of Graduate Studies, Department of Biopharmaceutical Sciences, University of Illinois, Chicago

11:45 AM-12:25 PM
Session VII: Panel Discussion: Nanomedicine: A global perspective

Panelists:
Ruth Duncan, PhD
Professor and Director, Center for Polymer Therapeutics, Cardiff University (Europe) 57

Kazunori Kataoka, PhD
Professor, Division of Clinical Biotechnology, Department of Materials Engineering, University of Tokyo (Japan) 59

Alexander (Sasha) Kabanov, PhD, DrSc
Parke-Davis Professor of Pharmaceutical Sciences and Director, Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center (USA) 61

Mansoor Khan, PhD
Director, Division of Product Quality Research, Center for Drug Evaluation and Research, Food and Drug Administration (USA) 63

Moderators:
Hamid Ghandehari, PhD
Associate Professor and Director, University of Maryland Center for Nanomedicine and Cellular Delivery

Kalle Levon, PhD
Professor, Associate Dean of Research and Intellectual Property, Polytechnic University

Each 5-10 min. perspective followed by discussion and questions from audience

12:25 PM-12:30 PM
Closing Remarks
Alexander (Sasha) Kabanov, PhD, DrSc
Parke-Davis Professor of Pharmaceutical Sciences and Director, Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center

Symposium ends-Turn in Evaluation forms / Dismantle posters (12:30-1:00 PM)

Poster Presentations

Abstract Number	Title and Presenter	Page
1	**In Vitro Evaluation Controlled Release Study For Metformin Hydrochloride Polymeric Hydrogel Matrices** E. M. Al-Zubaidi. Department of Chemistry, College of Science, University of Basrah, Basrah , Iraq.	66
2	**Development of a Novel Nano-Vesicle for the Treatment of Diseases with Inflammatory Component** Y. Avnir[1], P. Kizelsztein[1], Y. Naparstek[2], R. Ulmansky[2], Y. Barenholz[1]. [1]Laboratory of Membrane and Liposome Research, [2]Department of Medicine, Hebrew University-Hadassah Medical School, Jerusalem, Israel.	67
3	**Treating Head and Neck Cancer with Targeted Polymeric Conjugates** J. Boucek[1,2], J. Betka[2], J. Strohalm[3], D. Plocova[3], V. Subr[3], K. Ulbrich[3], B. Rihova[1]. [1]Institute of Microbiology, [2]Department of Otorhinolaryngology, Head and Neck Surgery, The First Medical Faculty, Charles University, University Hospital Motol, [3]Institute of Macromolecular Chemistry, Prague, Czech Republic.	68
4	**Animal Models for the Evaluation of Biodistribution and Efficacy of Polymer Therapeutics Targeting Solid Tumors** A.M. Burger[1], J.B. Schüler[2], H.H. Fiebig[2], E.A. Sausville[1]. [1]Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA, [2]Institute for Experimental Oncology, Freiburg, Germany.	69
5	**PLGA Nanoparticle-Aptamer Bioconjugates as Drug Delivery Vehicles for Targeted Prostate Cancer Therapy** J. Cheng[1†], B. A. Teply[1,2], I. Sherifi[1,2], E. Levy-Nissenbaum[1,2], A. Khademhosseini[3], R. S. Langer[1,3], O. C. Farokhzad[2-4]. [1]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, [2]Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [3]Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, [4]To whom correspondence should be addressed, [†]Current address: Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL.	70

Abstract Number	Title and Presenter	Page
6	**Synthesis and Characterization of Nanoparticulate Cap/Cisplatin for Lymphatic Targeted Drug Delivery** X. Cheng, L. T. Kuhn. Center for Biomaterials, University of Connecticut Health Center, Farmington, Connecticut, USA	71
7	**HPMA Copolymer-Doxorubicin Conjugates with pH-Controlled Activation; Effect of Hydrophobic Side Chains** P. Chytil, T. Etrych, C. Konák, K. Ulbrich. Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.	72
8	**Preparation and Characterization of PLGA Nanoparticles 9-Nitrocamptothecin, A Novel Anticancer Drug, by Nanoprecipitation Method** K. Derakhshandeh[1,2], G. Hochhaus[2], S. Dadashzadeh[1]. [1]Department of Pharmaceutics, School of Pharmacy, Shaheed Beheshti University, Tehran, Iran, [2]Department of Pharmaceutics, School of Pharmacy, University of Florida, Gainesville, USA.	73
9	**Computer-Aided Molecular Modeling -Trend Setting Approach in the Design of Bionano Drug Delivery Systems** G. S. Sonavane[1], M. Doble[2], P. V. Devarajan[1]. [1]Pharmaceutical Division, Mumbai University Institute of Chemical Technology, Mumbai, [2]IITM-Chennai, India.	74
10	**Extended Release of Hydrophilic Molecules from Vesicle-Biopolymer Gels** M.B. Dowling[1], J.H. Lee[1], G.F. Payne[2], S.R. Raghavan[1]. [1]Department of Chemical Engineering, [2]Center for Biosystems Research, University of Maryland, College Park, Maryland, USA.	75
11	**Prolonged Protection of Endothelium from Oxidative Stress by Targeting Antioxidant Enzyme-Loaded Polymer Nanocarriers** T. D. Dziubla[1], V. Shuvaev[1], S. Tliba[1], V.R. Muzykantov[1,2]. [1]Institute for Environmental Medicine, [2]Department of Pharmacology, School of Medicine; University of Pennsylvania, Philadelphia, Pennsylvania, USA.	76
12	**Enhanced Anti-Tumor Efficacy of Doxorubicin-Loaded Long-Circulating Liposomes Modified with Nucleosome-Specific Monoclonal Antibody 2C5** T. A. Elbayoumi, V. P. Torchilin. Department of Pharmaceutical Sciences, Bouvé College of Health Sciences, Northeastern University, Boston, Massachusetts, USA.	77

Abstract Number	Title and Presenter	Page
13	**Development and Evaluation of "Smart" Polymer-Antisense Oligodeoxynucleotide Complexes** M. E. H. El-Sayed[1], E. M. Bulger[2], A. S. Hoffman[1], P. S. Stayton[1]. [1]Department of Bioengineering, [2] Department of Surgery, University of Washington, Seattle, Washington, USA.	78
14	**HPMA Polymer Conjugates with Doxorubicin Attached Via Hydrazone Bond: Improvement of Efficacy** T. Etrych[1], P. Chytil[1], T. Mrkvan[2], B. Říhová[2], K. Ulbrich[1]. [1]Institute of Macromolecular Chemistry, [2]Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.	79
15	**Monodisperse Nanocarriers: Novel Fabrication of Polymeric Nanoparticles for Bionanotechnology** L. E. Euliss[1], C. M. Welch[2], B. W. Maynor,[1] J. P. Rolland,[1] J. M. DeSimone[1,2,3]. [1]Departments of Chemistry and [2]Pharmocology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, [3]Department of Chemical Engineering, North Carolina State University, Raleigh, North Carolina, USA.	80
16	**Nano-Scale Engineering at the Cell Surface: Synthesis and Delivery of Quorum Sensing Autoinducer at the Cell Surface Using Magnetic Nanofactories** R. Fernandes, W. E. Bentley. Bioengineering Program, University of Maryland, College Park, Maryland and Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, Maryland, USA.	81
17	**Monoclonal Antibody 2c5-Modified Liposomes Show Enhanced Accumulation in Subcutaneous Human Brain Tumor Xenograft in Nude Mice** B. Gupta, T. S. Levchenko, D. A. Mongayt, V. P. Torchilin Department of Pharmaceutical Sciences, Bouve College of Health Sciences, Northeastern University, Boston, Massachusetts, USA.	82
18	**Genetically Engineered Silk-Elastinlike Hydrogels for the Culture of Human Mesenchymal Stem Cells** M. Haider[1,2], H. Ghandehari[2], K. W. Leong[1]. [1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, [2]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, USA.	83

Abstract Number	Title and Presenter	Page
19	**Nano-Size Recombinant Polymer/DNA Complexes for Targeted Gene Delivery** A. Hatefi, H. Ghandehari. Department of Pharmaceutical Sciences, Center for Nanomedicine and Cellular Delivery, University of Maryland, Baltimore, Maryland, USA.	84
20	**Different Roles of Peptidic Spacers in Proteolytically and Hydrolytically Cleavable HPMA-Based Polymeric Prodrugs** O. Hovorka[1], L. Císlerová[1], J. Strohalm[2], V. Šubr[2], K. Ulbrich[2], B. Ríhová[1]. [1]Institute of Microbiology, [2]Institute of Macromolecular Chemistry, Prague, Czech Republic.	85
21	**Breaching the Blood Brain Barrier through Amino Acid Coupled Liposomes** N. K. Jain, A. Jain, P. Khare, V. Soni, A. Jain, Y. Gupta, S. K. Jain. Department of Pharmaceutical Sciences, Dr. Hari Singh Gour Vishwavidyalaya, Sagar, India.	86
22	**Poly (Amidoamine) Dendrimer Permeability and Cellular Localization in Caco-2 Cell Monolayers** K. M. Kitchens, P. W. Swaan, H. Ghandehari. Department of Pharmaceutical Sciences, Center for Nanomedicine and Cellular Delivery, University of Maryland, Baltimore, Maryland, USA.	87
23	**Oral Delivery of Insulin Plasmid Using Chitosan Nanoparticles** E. A. Klausner[1], E. Bachelder[2], P. Matzinger[2], K. W. Leong[1]. [1]Whitaker Biomedical Engineering Institute, The Johns Hopkins University, School of Medicine, Baltimore, Maryland, [2]Ghost Lab, Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.	88
24	**Preparation and Characterization of Antibody Labeled Magnetic Iron Oxide Nanoparticles for Bioseparations** I. Koh[1], X. Wang[2], B. Varughese[2], L. Isaacs[2], S. H. Ehrman[1], D. S. English[2]. [1]Department of Chemical Engineering, [2]Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland, USA.	89
25	**Sequential Changes in Salt Conditions During the HK: Plasmid Formation Markedly Augment Transfection Efficiency** Q. Leng, A. J. Mixson. University of Maryland Baltimore, School of Medicine, Baltimore, Maryland, USA.	90

Abstract Number	Title and Presenter	Page
26	**Physico-Chemical Characterization and Biological Evaluation of a Lipid-Based Formulation of a Hydrophobic Anti-Cancer Agent** J. Liu[1], M. Huesca[2], C. Allen[1]. [1]Faculty of Pharmacy, University of Toronto, [2]Lorus Therapeutics Inc. Toronto, Ontario, Canada.	91
27	**Influence of Serum Protein on Polycarbonate-Based Copolymer Micelles as a Systemic Delivery System for Hydrophobic Anti-Cancer Agent** J. Liu, F. Zeng, C. Allen. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.	92
28	**Polymer-Peptide Conjugates for Tumor Radiotherapy** A. Mitra[1,2], A. Nan[1,2], J. C. Papadimitriou[3], H. Ghandehari[1,2,4], B. R. Line[2,4,5]. [1]Department of Pharmaceutical Sciences, [2]Center for Nanomedicine and Cellular Delivery, [3]Department of Pathology, [4]Greenebaum Cancer Center, [5]Division of Nuclear Medicine, Department of Radiology, University of Maryland, Baltimore, Maryland, USA.	93
29	**ICAM-1-Targeted Nanocarriers Directed to Endothelial Cells** S. Muro[1,2], T. Dziubla[1], W. Qiu[1,3], J. Leferovich[1], X.Cui[1], E. Berk[1], V. R. Muzykantov[1,2]. [1]Institute for Environmental Medicine, [2]Department of Pharmacology, School of Medicine; [3]Department of Bioengineering, School of Engineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA.	94
30	**Enhanced Nuclear Import of Plasmid DNA and Increased Exogenous Gene Expression Using Streptavidin-Fused Importin-β** T. Nagasaki[1], T. Kawazu[1], S. Shinkai[2]. [1]Osaka City University, Graduate School of Engineering, Department of Applied and Bioapplied Chemistry, Osaka, [2]Kyushu University, Graduate School of Engineering, Department of Chemistry and Biochemistry, Fukuoka, Japan.	95
31	**Liposome Targeting of Combretastatin to Irradiated Tumors Results in Tumor Growth Control** C. B. Pattillo[1], R. C. Scott[1], B. Wang[1], D. Brown[2], P. L. Chong[2], M. F. Kiani[1,3]. [1]Department of Mechanical Engineering, [2]Department of Biochemistry, [3]Department of Radiation Oncology, Temple University, Philadelphia, Pennsylvania, USA.	96

Abstract Number	Title and Presenter	Page
32	**Nanostructures of Shed King Cobra Skin and Permeation of Parabens** A. Priprem[1], S. Pratontep[2], U. Rungsardthong[2], T. Pongjanyakul[1], P. Chitropas[1], C. Khamlert[1]. [1]Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen, [2]National Nanotechnology Center, National Science and Technology Development Agency, Pathumthani, Thailand.	97
33	**Characterization of Nanoparticles for Porosity and Fractal Dimension** S. Sant, P. Hildgen. Faculté de Pharmacie, Université de Montréal, Montréal, Canada.	98
34	**Targeting Liposomes to the Infarcted Cardiac Tissue** R. C. Scott[1], B. Wang[1], C. B. Pattillo[1], D. Brown[2] P. Chong[2], M. F. Kiani[1]. [1]Department of Mechanical Engineering, [2]Department of Biochemistry, Temple University, Philadelphia, Pennsylvania, USA.	99
35	**Shape and pH Determine Degradation Kinetics of PEG-PLA Polymer Nanocarriers** E. Simone[1,2], Y. Geng[1], F. Colon[3], D. Discher[1], V. R. Muzykantov[2,3], T. D. Dziubla[2]. [1]School of Engineering and Applied Sciences, [2]Institute for Environmental Medicine, [3]Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA.	100
36	**Loss of Elasticity of Aged Human Epithelial Cells In-Vitro and its Possible Recovery** I. Sokolov[1,2], S. Iyer[1], C. D. Woodworth[3]. [1]Department of Physics and [2]Chemistry, Clarkson University, Potsdam, New York, [3]Department of Biology, Clarkson University, New York, USA.	101
37	**MAXITARG-A Novel Targeting Approach for Hepatic Cancer** G. S. Sonavane, P. V. Devarajan. Pharmaceutical Division, Mumbai University Institute of Chemical Technology, Mumbai, India.	102
38	**Transcriptional Activation of Gene Expression by Pluronic Block Copolymers in Stably and Transiently Transfected Cells** S. Sriadibhatla, Z. Yang, A. V. Kabanov. Center for Drug Delivery and Nanomedicine, Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, Nebraska, USA.	103

Abstract Number	Title and Presenter	Page
39	**Semiconductor Nanocrystals and Biological Application** W. W. Yu, V. L. Colvin. Department of Chemistry, Rice University, Houston, Texas, USA.	104
40	**HPMA-Stabilized Long-Circulating DNA Nanoparticles with Sonoporation Enhanced Transfection** Q. H. Zhou[1], D. Soundara Manickam[1], D. L. Miller[2], D. Oupicky[1]. [1]Department of Pharmaceutical Sciences, Wayne State University, Detroit, Michigan, [2]Department of Radiology, University of Michigan, Ann Arbor, Michigan, USA.	105
41	**Assembly of Hydrogels with Controlled Protein - Delivery Profiles Via the Use of Peptide - Polysaccharide Interactions** L. Zhang[1], E. M. Furst[2], K. L. Kiick[1]. [1]Department of Materials Science and Engineering, [2]Department of chemical engineering, University of Delaware, Newark, Delaware, USA.	106

Speaker Abstracts and Biosketches

NON-VIRAL GENE TRANSFER: BASIC SCIENCE AND CLINICAL REALITY

F. C. Szoka

University of California, San Francisco, CA

Non-viral gene transfer is a clinical reality albeit not yet a therapeutic success. Thousands of patients have been exposed to various gene constructs leading to a plethora of positive, neutral as well as a few negative outcomes. GeneMedicine, Inc. was founded in 1993 on the belief that if gene therapy was to be a commonly used medical treatment in 2005, delivery would have to be simple and reproducible, the delivered gene would have to have a defined pharmacology and that genes would have to be re-administered, not be integrated into the genome. Clearly we were premature in our optimism for the technology of non-viral gene transfer. The paradigm in the gene transfer field had just been turned on its head by the discovery that naked DNA could transfect a wide variety of organs including: the muscle, liver and lung. Simple non-cationic polymers were identified that enhanced transfection or made the naked DNA phenomenon more reproducible. In spite of a variety of effective options for transferring genes in mice, a dozen years later there is no robust gene delivery vehicle, as opposed to physical technique, for robustly transferring genes in animals or humans after injection into the blood stream. My talk will present an overview of the current state of gene transfer, as opposed to gene therapy, in humans and other species and discuss one current paradigm where scientists are recapitulating the functions of viruses in an attempt to design a simple but robust carrier to transfer genes or other nucleic acid drugs after intravenous administration.

Supported by NIH NIBIB EB0003008

FRANCIS C. SZOKA

Francis C. Szoka is a Professor of Biopharmaceutical Sciences and Pharmaceutical Chemistry at the University of California, San Francisco. He directs a group that devises drug and gene carriers and examines their mechanism of action in cells and animals. His group studies liposomes, peptides and polymers. He received his Ph.D. in Biochemistry in 1976 from SUNY/Buffalo. He is the co-founder of Sequus, a liposome drug delivery company that created Doxil™ now owned by ALZA and of GeneMedicine, Inc., a gene therapy company, now known as Valentis, Inc.

SCRATCHING THE SURFACES: NANOTECHNOLOGY IN THE REAL WORLD

M. C. Davies, S. J. B. Tender, P. M. Williams, C. J. Roberts, S. Allen

Laboratory of Biophysics & Surface Analysis, School of Pharmacy, University of Nottingham, Nottingham
E-mail: Martyn.Davies@nottingham.ac.uk Website: www.nottingham.ac.uk/lbsa

The characterization of the surface structure of both conventional and advanced biomedical systems can be an important step in understanding the performance and optimizing the function of such healthcare devices. A number of advanced biophysical analytical techniques have emerged for the study of pharmaceutical and biomedical systems. In this talk, we shall explore the role of scanning probe microscopy, in connection with complimentary techniques, in the study of surface structure and function of advanced polymeric materials. The visualization of surface topography and morphology of polymeric devices will be discussed and will include the condensation of polymeric constructs for gene therapy to the single molecule imaging of micro-patterned proteins on nanoengineered tissue-engineering substracts. The role of the force microscope in determining interparticulate and inter-molecular forces in order to explore its potential for the study of biomolecular interactions and polymer interfaces through to the macromolecular stimuli response hydrogels. The potential of the biophysical methodology of high-resolution imaging and force spectroscopy to aid research in biorecognition, development of gene delivery systems and understanding interparticulate and molecular forces, will be highlighted. The talk will encourage a comprehensive approach for characterization of complex pharmaceutical systems and look at future opportunities.

MARTYN DAVIES

Martyn Davies is Professor of Biomedical Surface Chemistry and Director of The Laboratory of Biophysics and Surface Analysis (LBSA) at the School of Pharmacy, University of Nottingham, leading a team of 5 academics. The LBSA is home to a multidisciplinary academic group of 30 PhD students and Postdoctoral Fellows providing novel insights into nanoscale structure, function and interactions of biological, biomedical and pharmaceutical interfaces. Activities include the measurement and simulation of molecular forces that underpin receptor/ligand interactions and protein folding. Surface analytical tools are used for the characterization of advanced biomedical materials, including tissue engineering scaffolds. Studies on the dynamic surface properties of drug crystals and interparticulate interactions demonstrate a strong interface with the pharmaceutical industry. Novel instrumentation is also being developed, such as intra-capillary optical trapping approaches for single cell metabolomic studies. The LBSA is a European Union Marie-Curie Training Site and was awarded the 2003 GlaxoSmithKline International Achievement Award. More details of the LBSA activities and facilities can be seen at www.nottingham.ac.uk/lbsa. Professor Davies obtained his PhD in Pharmacy at the Chelsea School of Pharmacy, University of London. He joined the School of Pharmacy at Nottingham in 1985 and obtained a personal chair in 1996. Professor Davies served as the Head of School of Pharmacy from 2000-2003. He is currently Scientific Secretary of the Controlled Release Society. He is a Fellow of the Royal Pharmaceutical Society and the Royal Society of Chemistry. Professor Davies has supervised over 60 PhD students to successful completion of their PhD, many of whom have gone on to postdoctoral fellowships, many hold prominent posts within the Pharmaceutical, Chemical, Polymer & Diagnostics Industries and one has moved successfully into Pharmacy Management. His first student is now Director for Drug Delivery of a multinational pharmaceutical company. Professor Davies has also co-supervised over 35 postdoctoral fellows and 15 of these have moved onto academic positions in University Science (7) and Pharmacy (8) Departments and five hold personal chairs (Professors). Professor Davies has published over 300 scientific papers and reviews.

RECOMBINANT POLYMERS AS PLATFORMS FOR NANOCONSTRUCTS

K. L. Kiick

University of Delaware, Department of Materials Science and Engineering

In order to develop materials that can elicit specific responses to chemical and biological stimuli, it has become increasingly important to understand critical design features that control the structure, function, and assembly of macromolecules. Such understanding may permit the design of novel and functional biomolecular structures that are capable of selectively and efficiently interacting with cellular and other targets and/or directing materials properties. In the Kiick group, genetically directed methods are being employed to produce artificial repetitive proteins capable of controlled presentation of ligands such as saccharides and peptides. The well-defined protein polymers produced via these methods exhibit desired and controlled conformational behavior and are being used to study biological phenomena such as the role of glycopolymer architecture in mediating biological binding events and to explore protein-protein interactions in the assembly of well-defined materials constructs. We are also utilizing protein/polymer conjugates to probe the use of biologically relevant protein-saccharide interactions as a mechanism for controlling network formation and degradation in drug delivery matrices. Significant opportunities exist for utilizing these architectures for understanding mechanisms of cellular interactions with materials and for developing networks with controlled properties useful for biomaterials applications. Ultimately, our goals are not only to understand the macromolecular structure-function relationships that govern the biological responses of materials, but also to produce macromolecules with uniquely optimized properties for applications in biology and medicine.

KRISTI L. KIICK

Kristi Kiick is an Assistant Professor of Materials Science and Engineering at the University of Delaware and joined the faculty in August 2001. Her doctoral degree in Polymer Science and Engineering was awarded from the University of Massachusetts Amherst in 2001. Her doctoral research was conducted at the California Institute of Technology under the direction of David Tirrell and involved expanding the synthetic versatility of protein engineering by the in vivo incorporation of non-natural amino acids into proteins. Prior to her doctoral program, Kiick's industrial work experience included four years in research and development at Kimberly Clark Corporation, where she developed benign protein-based methods for the surface functionalization of polypropylene nonwoven fabrics. Her current research programs are focused on combining biosynthetic techniques, chemical methods, and bioinspired assembly strategies for the production of novel protein-polymer architectures with advanced multifunctional behaviors. These research programs are funded in part by a Camille and Henry Dreyfus Foundation New Faculty Award, a Beckman Young Investigator Award, an NSF CAREER Award, and a DuPont Young Professor Award.

DELIVERY SYSTEM FOR REMEDIATION OF CELLULAR HYPOXIC DAMAGE

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INTRODUCTION

Many known pathological conditions lead to decreases in oxygen supply to various cells. When secondary cellular hypoxia becomes severe, it causes additional cellular damage, aggravating the primary disorder and leading to cell death. Therefore, remediation of secondary hypoxic damage should significantly increase the efficacy of the treatment of primary disease and prevent extensive cellular damage. It was found that c-jun N-terminal kinase 1 (JNK1) plays a central role in the development of tissue damage under hypoxia [1-5]. We hypothesized that suppression of JNK1 will decrease hypoxic cellular damage and might increase the efficacy of traditional treatment of many pathological conditions [6]. The present investigations are aimed at studying the influence of the suppression of JNK1 on the development of cellular hypoxic damage.

EXPERIMENTAL METHODS

We proposed a novel antihypoxic delivery system (DS) [6] which contains antisense oligonucleotides (ASO) or siRNA targeted to JNK1 mRNA to inhibit the translation step and the synthesis of corresponding protein. Experiments were carried out on human kidney cells under normoxic and hypoxic conditions. Neutral or cationic liposomes were used as carriers for DS [7]. Mechanisms of hypoxic cellular damage were studied.

RESULTS AND DISCUSSION

Designed DS provided effective delivery of ASO or siRNA into cell nuclei and targeted JNK1 protein was suppressed. Hypoxia led to lactate accumulation and induced cell death by apoptosis and necrosis. The suppression of JNK1 in normoxic conditions did not result in significant changes in cellular metabolism. In contrast, the blockade of JNK1 protein under hypoxia substantially decreased hypoxic cellular death mainly by the limitation of caspase-dependent apoptotic signal.

CONCLUSIONS

The results suggest that the suppression of JNK1 may substantially decrease hypoxic cellular damage and therefore may be used to increase the efficacy of treatment of many diseases accompanied by cellular hypoxia.

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TAMARA MINKO

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Education/Training:
M.S. – Biochemistry, Ph.D. – Cellular and Molecular Physiology (Kiev, Ukraine).
Postdoctoral training: Molecular and Cellular Biology and Pharmaceutics (University of Utah, Salt Lake City, Utah).

Current Research Interests:
Drug delivery; biopharmaceutics; molecular targeting; antisense oligonucleotides, siRNA and peptides in cancer therapy; mechanisms of multidrug resistance; intracellular fate and molecular mechanisms of action of anticancer drugs: apoptosis and necrosis, signal transduction, DNA repair, replication and biosynthesis, antiapoptotic cellular defensive mechanisms; use of macromolecules for drug delivery; preclinical evaluation of anticancer drugs; cell death mechanisms during hypoxia.

Selected Publications:
Publications selected from 68 journal research articles, 14 book and textbook chapters, 34 extended abstracts, 137 abstracts.

1. S. S. Dharap, Y. Wang, P. Chandna, J. J. Khandare, B. Qiu, S. Gunaseelan, P. J. Sinko, S. Stein, A. V. Farmanfarmanian, **T. Minko**, Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide, Proc. Natl. Acad. Sci. USA, 102, 12962-12967 (2005).
2. **T. Minko**, Y. Wang, V. Pozharov, Remediation of cellular hypoxic damage by pharmacological agents, Curr. Pharm. Des., 11, 3185-3199 (2005).
3. R. I. Pakunlu, Y. Wang, W. Tsao, V. Pozharov, T. J. Cook, **T. Minko**, Enhancement of the efficacy of chemotherapy for lung cancer by simultaneous suppression of multidrug resistance and antiapoptotic cellular defense: novel multicomponent delivery system, Cancer Research, 64, 6214-6224 (2004).
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7. **T. Minko**, Drug targeting to the colon with lectins and neoglycoconjugates, Adv. Drug Deliver. Rev., 56, 491-509 (2004).
8. S. S. Dharap, **T. Minko**, Targeted proapoptotic LHRH-BH3 peptide, Pharm. Res., 20, 889-896 (2003).

NANOSYSTEMS BIOLOGY WITH APPLICATIONS TO *IN VITRO* AND *IN VIVO* DIAGNOSTICS

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The picture of cancer is evolving into one in which similar clinical presentations are now being stratified into different and distinct diseases, each potentially with its own prescribed molecular therapies. The implication is that therapeutics and diagnostics will have to become increasingly coupled. In vitro diagnostics will include technologies that are capable of quantitating large numbers of genomic or proteomic markers, and in vivo diagnostics (molecular imaging) will require an expanded molecular tool set of imaging probes. The goal of both is to identify the presence and specific molecular identify of the disease, the progression of the disease, the positive and adverse responses of the disease to therapy. In this talk, I will discuss a host of technologies that I and my collaborators are working on to achieve this goal. These technologies begin with a systems biology view of the disease in which comprehensive genomic and proteomic measurements are utilized to catalogue a cancer. From this data base we identify relatively large panels of organ specific, secreted biomarkers that can be utilized for in vitro diagnostics. We also identify up- or down-regulated metabolic processes that can be exploited for in vivo molecular imaging. For both cases, nanotechnology tools, new materials, and new chemical technologies are being brought together to build platforms for a quantitative, real-time multiparameter analysis of serum proteins, the preparation of high-affinity protein capture agents, and the rapid preparation of new in vivo molecular imaging probes. Validation of some of these technologies using either serum samples or mouse models of cancer will be presented, and development timelines for many of these new technologies will also be discussed.

JAMES R. HEATH

James R. Heath is the Elizabeth W. Gilloon Professor and Professor of Chemistry at Caltech, and Professor of Molecular & Medical Pharmacology at the David Geffen School of Medicine at UCLA. Heath received a B.Sc. degree in 1984 (Baylor) and his Ph.D. in Chemistry (Rice) in 1988 where he was the principal student involved in the Nobel Prize–winning discovery of C_{60} and the fullerenes. Heath was a Miller Fellow at UC Berkeley from 1988-91, and on the Technical Staff at IBM Watson Labs from 1991-93. In 1994 he joined the faculty at UCLA. He founded the California NanoSystems Institute in 2000 and served as its Director until moving to Caltech. Heath has investigated quantum phase transitions, and he has developed architectures, devices, and circuits for molecular electronics, and has founded or co-founded 4 companies. His group has recently been applying their nanoelectronics and microfluidics technologies towards addressing problems in cancer. He has received a number of awards, including a Public Service Commendation from Governor Grey Davis, the Sackler Prize, the Spiers Medal, the Feynman Prize, the Jules Springer Prize, and the Arthur K. Doolittle Award.

SMART DELIVERY SYSTEMS FOR BIOMOLECULAR THERAPEUTICS

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INTRODUCTION

A hallmark of many biomolecular machines is the ability to change their structural and functional properties in response to specific environmental signals. An important example relates to the molecular mechanism underlying the potent ability of viruses and pathogens to gain entry to the cytoplasm of target cells. Specific proteins sense the lowered pH gradient of the endosomal compartment and are activated to destabilize the endosomal membrane, thereby enhancing protein or DNA transport to the cytoplasmic compartment. These molecular mechanisms provide interesting paradigms for the development of new polymeric delivery systems that mimic biological strategies for promoting the intracellular delivery of biomolecular drugs. The key feature of these polymers is their ability to directly enhance the intracellular delivery of proteins and DNA, by destabilizing biological membranes in response to vesicular compartment pH changes.[1] The ability to deliver a wide variety of protein and nucleic acid drugs to intracellular compartments could open new drugs and drug targets in a variety of therapeutic applications.

EXPERIMENTAL PROCEDURES

A pyridyl disulfide acrylate monomer was synthesized following the method reported earlier to carry biomolecular drugs (17). The first series of PDSA-containing polymers was prepared by free radical polymerization of PDSA monomers with different pH-sensitive monomers including methyl(acrylic acid), ethyl(acrylic acid) and propyl(acrylic acid) using AIBN as an initiator. The molar feed ratio of PDSA and pH-sensitive monomers was adjusted to 5 % and 95 %, respectively. The second series of PDSA-containing polymers incorporated the hydrophobic butyl acrylate (BA) monomer with the PDSA and pH-sensitive monomers utilized in the first series. The molar feed ratio of PDSA, BA, and pH-sensitive monomers was adjusted to 5 %, 25 %, and 70 %, respectively. ^{1}H-NMR spectroscopy was used to confirm the purity of the synthesized polymers and to examine their compositions.

RESULTS & DISCUSSION

The pH-responsive PDSA compositions are designed for: a) reversible destabilization of the endosomal membrane and diffusion of the carrier-drug system into the cytoplasm at endosomal pH, and b) release of the disulfide-conjugated drug molecules into the cytoplasm by the reducing action of glutathione or redox enzymes, commonly present in the cytosol. We have examined the influence of composition of PDSA-containing polymers on their pH-sensitive membrane-destabilizing activity.[2,3] The pH-dependence and hemolytic activity depends on the length of the hydrophobic alkyl group substituted on the pH-sensitive monomer. Relatedly, the addition of a hydrophobic monomer such as BA can also tune the pH-dependence toward higher pH transitions and higher hemolytic activity. This research has produced several promising pH-sensitive, membrane-destabilizing, and glutathione-reactive polymer compositions such as poly(PAA-*co*-PDSA), poly(EAA-*co*-BA-*co*-PDSA), and poly(PAA-*co*-BA-*co*-PDSA) polymers. These compositions have been used to deliver antisense oligonucleotides and siRNA drugs in anti-inflammation and anti-cancer applications.

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PATRICK STAYTON

Dr. Stayton serves as the Washington Research Foundation endowed Professor in the Dept. of Bioengineering at the University of Washington, where he directs the Molecular Materials group at the UW Engineered Biomaterials Center. He has been elected as a Fellow of the American Institute for Medical and Biological Engineering, and has received the Controlled Release Society's-Cygnus Recognition Award, the Clemson Hunter Visiting Professorship, Kobe University Visiting Professorship, and the UW Minority Science Engineering Program Award. His current research interests are in the areas of drug delivery and diagnostics technology development, biomaterials, tissue engineering, biomineralization and molecular recognition.

ICS-283: TISSUE-TARGETED SIRNA NANOPARTICLE ANTI-ANGIOGENESIS THERAPEUTIC – DUAL TARGETING AND MULTITARGETED COCKTAIL

M. C. Woodle

Chief Scientific Officer, Intradigm Corporation

Short dsRNA oligonucleotides, called siRNA, are the potent active intermediate of the recently discovered RNA Interference process, an endogenous mechanism of gene inhibition. The use of siRNA has proven to be a robust means to inhibit genes with a high degree of selectivity based solely on the gene sequence, promising to enable a further revolution in "targeted" therapeutics. Intradigm has developed tissue-targeted nanoparticle delivery systems for siRNA as a means to address the many barriers to systemic administration of these dsRNA oligonucleotides as therapeutics. The combination of tissue selective nanoparticle delivery with gene selective siRNA inhibitors opens the door to dual-targeted therapeutics. Intradigm is developing a first product in this class, ICS-283, for inhibition of neovascularization and angiogenesis. In addition, Intradigm's siRNA nanoparticles have been shown to permit cocktails of siRNA blocking multiple therapeutic targets simultaneously, what is called "multitargeted" therapeutics, giving a greater impact on the pathology. The overall science of these revolutionary capabilities of Intradigm's tissue-targeted nanoparticle siRNA therapeutics will be described in general and specifically the properties of Intradigm's lead product, ICS-283.

MARTIN C. WOODLE

Dr. Martin C. Woodle has over 20 years experience in pharmaceutical research and development. He currently serves as Chief Scientific Officer and co-founded Intradigm Corporation, a biotechnology company developing therapeutics based on nucleic acid delivery technology originally developed while he was Director Synthetic Gene Vectors at Novartis Genetic Therapy Inc. Prior to his position in Novartis, Dr. Woodle was Director of Formulations and Drug Delivery at Genta Inc., a biotechnology company developing antisense therapeutics, where he was involved in formulation and preclinical studies on Genasense (G-3139). Earlier, Dr. Woodle was appointed to lead the Basic Technology department of Liposome Technology, Inc. (later known as Sequus and acquired by Alza). While at LTI, Dr. Woodle led the discovery and development PEG-PE based Stealth® Liposomes and their use for cancer and infectious disease treatments including Alza's Liposomal Doxorubicin or Doxil®, and he led implementation of research that generated a product pipeline. Dr. Woodle obtained his Ph.D. in Biochemistry, Molecular and Cell Biology at Northwestern University and continued his academic studies at The Rockefeller University.

TEMPLATE-SYNTHESIZED MAGNETIC NANOTUBES FOR DRUG DELIVERY.

S. B. Lee

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Tubular structure of nanoparticle is highly attractive due to their structural attributes, such as the distinctive inner and outer surfaces, over conventional spherical nanoparticles. Inner voids can be used for capturing, concentrating, and releasing species ranging in size from large proteins to small molecules. Distinctive outer surfaces can be differentially functionalized with environment-friendly and/or probe molecules to specific target. Magnetic particles have been extensively studied in the field of biomedical and biotechnological applications, including drug delivery, biosensors, chemical and biochemical separation and concentration of trace amount of specific targets, and contrast enhancement in magnetic resonance imaging (MRI). Therefore, by combining the attractive tubular structure with magnetic property, the magnetic nanotube (MNT) can be an ideal candidate for the multifunctional nanomaterial toward biomedical applications, such as targeting drug delivery with MRI capability. Here, we successfully synthesized magnetic silica-iron oxide composite nanotubes and demonstrated the magnetic-field-assisted chemical and biochemical separations, immunobinding, and drug delivery.

SANG BOK LEE

Dr. Sang Bok Lee is an assistant professor at the Department of Chemistry and Biochemistry, University of Maryland (UMD), College Park, MD. He received his BS in Chemistry and MS in physical chemistry (SERS) and PhD in physical organic chemistry (molecular recognition) from Seoul National University, Korea. After finishing his PhD, he worked at a DRAM maker, LG Semicon (Hynix), for two years as senior research engineer and held a postdoctoral position at the University of Florida, before joining UMD in 2002. His research interests includes electrochemical synthesis of nanotube structure, fast electrochromics with nanotube structures, magnetic nanotubes for bioimaging and drug delivery, nanoscale single channel fabrication for sensor, and molecular transport and diffusion properties of nanotubes and nanotube membranes.

DENDRIMERS – A PROMISING APPROACH TO TAILORED CARRIERS IN DRUG DELIVERY APPLICATIONS

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INTRODUCTION

40% of potential drugs are rejected by the pharmaceutical industry because of their poor water solubility, and approx. 17% of launched drugs exhibit suboptimal performance due to their low bioavailability. Drugs need carriers to improve their bioavailability – by enhancing either their water solubility or their membrane permeability. In addition, carriers can provide the option of targeted delivery, i.e., passive targeting through size exclusion (Enhanced Permeability and Retention (EPR) Effect) or active targeting through ligands such as folic acid that interact with receptors overexpressed on tumor cell surfaces, e.g. the high affinity folate receptor, hFR. Successful drug carriers should, therefore, increase the bioavailability of a drug, provide site-selective delivery, and reduce drug side effects such as cytotoxicity. Poly(amidoamine) (PAMAM) STARBURST® dendrimers are a class of core-shell nanostructures with precise architecture and very low polydispersity. These nanostructures are being synthesized in a layer-by-layer fashion around a core unit, resulting in a high level of control over size, branching points and surface functionality. This high level of control of dendrimer architecture, and the resulting ability to tailor dendrimers to the needs of a drug, makes them ideal carriers for drug delivery applications. Examples will be presented to substantiate this observation, two of them being the anti-cancer drug cisplatin and the non-steroidal anti-inflammatory drug (NSAID) indomethacin.

EXPERIMENTAL METHODS

Cisplatin and indomethacin have been encapsulated into STARBURST® dendrimers, and their encapsulation efficiency and release profiles in DI water and PBS at pH 7.2 have been studied. The drugs were dissolved in DI water under heating and ultrasonication, and then cooled to ambient temperature. An aqueous dendrimer solution was added, and the mixture kept at ambient temperature under stirring for 22 hours. Non-encapsulated cisplatin was removed by Amicon plus-80 centrifugal filter and the dendrimer-drug complex isolated and lyophilized. The platinum content was measured by atomic absorption (AA), while the indomethacin content was measured by UV absorption.

RESULTS AND DISCUSSIONS

Cisplatin. Encapsulation was only successful using dendrimers with carboxylate (COO^-) surface, while the dendrimers size (generations 3.5 or 4.5) and size of the core molecule (C2, C4, or C12) had little effect on the encapsulation efficiency. The release profiles in DI water and PBS revealed a two-step process, a burst release over 30 minutes, followed by a sustained release over several hours. Both release profiles were very similar despite the higher ionic strength in PBS; however, the release in PBS was shifted to slightly higher percentages.

Indomethacin. Encapsulation was successful in dendrimers with amino (NH_2), hydroxy (OH) and carboxylate (COO^-) surfaces with NH_2>OH>COO^-. As expected, the encapsulation efficiency increased with dendrimer size; however, the core size (C2 vs. C12) had little effect. The release profiles were dependent on the surface groups (NH_2<OH<COO^-), the dendrimer size (G3>G4>G5>G6) and the size of the core molecule (C2>C12).

For both drugs, encapsulation enhanced their water solubility by orders of magnitude. The cytotoxicity of cisplatin was greatly reduced through its encapsulation, as shown on several cell lines (B16F10, murine melanoma cells; CCRF-CEM, lymphoblastic leukemia cells; HepG2, hepatocellular carcinoma cells; and Caco-2, human colon adenocarcinoma cells).

CONCLUSIONS

PAMAM STARBURST® dendrimers are a versatile platform that can be tailored to the needs of a drug to achieve the desired drug encapsulation efficiency and release profile.

SONKE SVENSON

Dr. Svenson received his Ph.D. degree in Organic and Macromolecular Chemistry from the Free University Berlin, Germany. He held postdoctoral research positions at Purdue University, Northwestern University, and Princeton University funded by NSF, NIH, and industry, which focused on the synthesis and self-assembly of lipids and surfactants for biomedical and technical applications. Dr. Svenson joined The Dow Chemical Company as a Research Specialist (2000), conducting research to improve the water solubility, and therefore, bioavailability of poorly water soluble drugs. In 2003, he joined Dendritic NanoTechnologies, Inc. (DNT) as a Senior Scientist, leading DNT's research efforts in drug delivery, i.e., utilizing the dendrimer platform in targeted delivery, controlled/sustained release of pharmaceuticals and enhanced drug solubilization. Dr. Svenson is an editorial board member of the journal Drug Delivery. Recently, he organized two international symposia sponsored by the American Chemical Society (ACS), which focused on drug delivery. He was a co-organizer and the Scientific Program Coordinator of the 4th International Dendrimer Symposium (2005). Dr. Svenson is the editor of three ACS Symposium Series books entitled: "Carrier-Based Drug Delivery" and "Polymeric Drug Delivery: Science & Application, Volumes I and II" (in press). Dr. Svenson is the author/coauthor of 30 peer-reviewed publications/book chapters and four patent applications.

DRUG DELIVERY USING NANOPARTICLES WITH NOVEL MORPHOLOGIES

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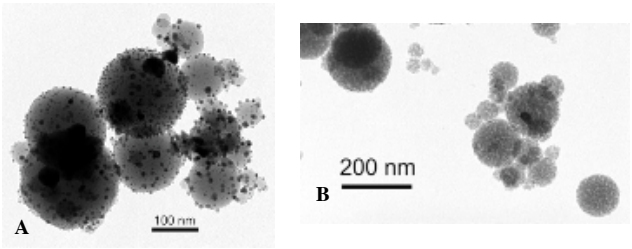
INTRODUCTION

Nanoparticles possessing novel morphologies should be ideal vehicles for drug delivery. Particularly attractive morphologies would include porous nanomaterials and phase segregated nanoparticles of two or more components (ie. gold and silica). A number of challenges must be addressed if these materials are to serve as the basis of drug delivery vehicles: (1) the synthesis of nanoparticles with novel morphologies with control of dispersity, surface area, and composition, (2) development of nanoparticle surface functionalization chemistry that allows for the attachment of robust and effective targeting entities, (3) the ability to differentially functionalize surfaces of nanoparticles having more than one component, and (4) knowledge of the diffusional characteristics of nanomaterials, particularly porous nanoparticles.

RESULTS

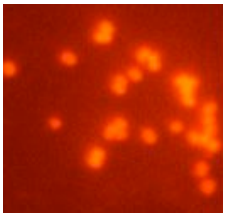
We have prepared nanomaterials with a variety of unusual morphologies including porous silica (B, Figure 1), silica into which gold nanoparticles have been embedded, and silica in which gold nanospheres are on the surface of the silica particle (A, Figure 1). The techniques developed for the synthesis of these novel nanoparticles can be applied to the synthesis other composites such as silica-silver, alumina-gold/silver, silica/alumina-iron.

Figure 1



Surface functionalization of nanoparticles has been achieved using a variety of bioconjugates including thiol and siloxane derivatives of either oligosaccharide-based cell surface receptor ligands and peptide-based cell surface ligands. The viability of this strategy has been demonstrated by showing that the surface functionalized particles have specific binding with both enzymes (lectins) and cells *in vitro* (Figure 2).

Figure 2



Luminescent gold nanoparticles attached to *Neisseria*

Finally, we have also begun studies designed to probe the rates of diffusion of these porous nanomaterials. The diffusion studies are particularly important because the rate of drug loading and release has to be determined if effective drug delivery is to be achieved.

The application of these methods for development drug delivery vehicles will be discussed.

PHILIP DESHONG

Philip DeShong received his undergraduate training at the University of Texas, Austin and his doctorate from the Massachusetts Institute of Technology under the direction of George Buchi. After postdoctoral studies at the ETH, Zurich and MIT, he began his academic career at the Pennsylvania State University. In 1980, he moved to the University of Maryland and is currently Professor of Chemistry and Biochemistry and is a member of the Bioengineering Program.

His long term research interests have been in the synthesis of natural products and the development of synthetic methodology. More recently, his group has been interested in the synthesis and reactivity of siloxane derivatives, surface functionalization techniques using biomolecules, and biosensor development.

Professor DeShong is a University of Maryland Distinguished Scholar-Teacher and is a Fellow of the American Association for the Advancement of Science. He has been a DuPont Faculty Fellow, American Cyanamid Science Faculty Fellow, and held the Swiss Chemical Society Lectureship. He is also currently the Chemistry Judge for the Intel Science Talent Institute.

POLYMERS AND POLYMERIZATON IN MOLECULAR IMAGING

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There are two major approaches to polymeric probes use for molecular imaging:
1. Imaging signal can be "encoded" in a polymer molecule in a quenched form to enable a release of the signal upon the interaction with hydrolases that cleave the polymer backbone. We previously synthesized and tested of a number of protease-specific polymers as *in vivo* imaging probes. One lead compound with a potential for further translation into clinical testing is cathepsin B/L/H sensing near-infrared fluorescent (NIRF) polymeric probe. This probe has been originally designed as a non-immunogenic biocompatible, protected graft copolymer (PGC) and tested in normal volunteers. The molecule showed a long circulation time resulting in accumulation in tumors. The NIRF prototype probe has been used to detect early breast and gastrointestinal tumors and has shown improvements in the detection of tumors and facilitated imaging of early dysplastic lesions. Cathepsins are involved in several key processes of tumor progression including: a) extracellular matrix remodeling, b) invasion and metastasis and c) *in vivo* tumor cell endocytosis. Therefore, we performed further identification of cells that participate in enzymatic de-quenching of fluorescence of cathepsin-specific probes by using *in vivo* microscopy. 2. Polymerization of monomers into polymers under the conditions of enzyme-mediated catalysis can also be used in imaging. We previously reported a class of "amplifiable" paramagnetic substrates that polymerize in the presence of oxidoreductases (peroxidase and myeloperoxidase, MPO). Physical characterization of these substrates showed that polymerized agent exhibited a 3-times higher relaxivity than the monomeric agent, making it a potential reporter of enzymatic activity. Computer simulations of NMR relaxometry profiles showed that the rise in effective atomic relaxivity was due to a 9-times increase in the rotational correlation time (τ_R). Decreased inner-sphere water residence lifetime limited the effectiveness of τ_R increase. The same amplification principle was suggested as a potential strategy for detecting local MPO activity levels in vulnerable plaques. Several potential substrates for MPO were synthesized, tested and yielded one lead compound - a covalent conjugate of GdDOTA and serotonin. The obtained paramagnetic substrate efficiently polymerized in the presence of human neutrophil MPO resulting in a 70-100% increase of relaxivity. As a result, MPO activity could be imaged in Matrigel phantoms containing MPO and glucose oxidase (the source of hydrogen peroxide), as well as in mouse models harboring MPO-containing implants. Therefore, amplification effects based on enzyme-mediated relaxivity changes in paramagnetic chelates are highly feasible *in vitro* and *in vivo*. The application of these approaches for in vivo imaging enables widening the repertoire of potential molecular targets that could be detected with an aid of non-invasive imaging modalities.

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ALEXEI A. BOGDANOV, JR., PH.D.

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Moscow State University, Moscow, USSR	M.Sci.	1983	Chemistry
All-Union Cardiology Research Centre, Moscow, USSR	Ph.D.	1988	Biochemistry and Cell Biology

EMPLOYMENT, HONORS (From 1990)

1990	Visiting Scientist, Max-Planck-Institute for Experimental Medicine, Goettingen, Germany
1990-91	Fellow, Webster Center for Biological Science, Amherst College, Amherst, MA
1991-93	Research Fellow, Department of Radiology, Massachusetts General Hospital, Boston, MA
1994	Controlled Release Society Inc. Outstanding Pharmaceutical Paper Award
1993-96	Research Instructor, Assistant in Chemistry, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
1996-1999	Assistant Professor of Radiology, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
1997-	Fuji Film/RSNA Award
1999-	ISMRM Young Investigators' Rabi Award Paper
1999-2006	Associate Professor of Radiology, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
2004-	The Academy of Molecular Imaging Top Basic Science Abstract Award
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PEPTIDE-POLYMER NANOHYBRIDS FOR ANGIOGENESIS TARGETED CANCER RADIOTHERAPY

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INTRODUCTION: Tumor angiogenesis is common to all solid tumors. CDCRGDCFC (RGD4C) peptide is a ligand of the $\alpha_V\beta_3$ integrin expressed on endothelial cells of angiogenic tumor vessels. We tested the hypothesis that a ^{99m}Tc *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-multivalent RGD4C conjugate (HPMA-RGD4C) would show significantly better targeting biokinetics than ^{99m}Tc RGD4C peptide alone and that alpha and beta radiotherapy could be delivered via the nanohybrid in sufficient quantity to arrest tumor growth.

METHODS: Biodistribution studies of ^{99m}Tc HPMA-RGD4C and ^{99m}Tc RGD4C in SCID mice bearing xenografts of DU145 human prostate carcinoma were compared to a control copolymer ^{99m}Tc HPMA-RGE4C (RGE4C has no $\alpha_V\beta_3$ affinity) and ^{99m}Tc RGE4C. Copolymer RGD4C/RGE4C conjugates were synthesized containing cyclohexyl-diethylenetriaminepentaacetic acid (CHX-A-DTPA) for ^{90}Y and ^{210}Po labeling. The conjugate was characterized by its molecular weight, side chain content, radiochemical purity and bioreactivity (by HUVEC adhesion). The biodistribution of ^{99m}Tc HPMA-RGD4C conjugates was assessed in SCID mice bearing xenografts of DU145 human prostate carcinoma. Imaging at 1, 24 and 48 h post-injection was followed by necropsy and organ counting. HPMA-RGD4C was injected in groups of 6 mice at ^{90}Y dose levels of 100 μCi and 250 μCi and ^{210}Po dose levels of 5 μCi, 1 μCi and 0.2 μCi followed by daily tumor size measurements and histopathological analysis at 21 days post-injection.

RESULTS: There were approximately 15 RGD4C peptides (0.49 mmol/g polymer) and 16 RGE4C peptides (0.51 mmol/g polymer) per copolymer. HUVEC studies showed inhibition of cell adhesion with HPMA-RGD4C and RGD4C, but no inhibition by HPMA-RGE4C, RGE4C or unmodified HPMA. Necropsy counts showed higher tumor accumulation of RGD4C compounds relative to RGE4C ($p<0.001$) and higher copolymer-peptide localization relative to free peptide ($p<0.05$). Whereas the ^{99m}Tc labeled conjugate was not retained by any body tissue, there was a continuous increase in tumor localization to 4.32 ±0.32% ID/g at 72 h. For 7 days following treatment, tumor volume decreased in all treatment groups relative to controls for ^{90}Y polymer. For ^{210}Po polymer (Rx volume/Control at 7 days: H=0.19, M=0.26, L=0.28) but only the high dose group continued to decrease by 14 days. ^{90}Y copolymer treated tumor showed increased apoptosis, higher numbers of thanatosomes and more pronounced nuclear atypia as compared to the control histopathology.

CONCLUSIONS: Tumor neovasculature can be specifically targeted using multivalent peptide polymeric conjugates of RGD4C. ^{210}Po and ^{90}Y labeled *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-RGD4C conjugate may provide molecularly guided high LET alpha or beta radiotherapy through a vascular target common to all solid tumors.

Acknowledgements. NIH Grants (CA 99015 and 98008).

BRUCE R. LINE

Professor of Radiology University of Maryland School of Medicine and Director of the Division of Nuclear Medicine at the University of Maryland Medical Systems. He is also a member of the Greenebaum Cancer Center at the University of Maryland School of Medicine, and is one of the initiating members of the Center for Nanomedicine and Cellular Delivery at University of Maryland School of Pharmacy.

Dr. Line and Dr. Ghandehari have been collaborating on the delivery of nanohybrid polymer-peptide conjugates to sites of tumor angiogenesis.

MULTIFUNCTIONAL NEAR-INFARED NANOPARTICULATE SYSTEM FOR DIAGNOSIS AND THERAPY

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INTORUDCTION:

The objective of this study is to develop a stable, biodegradable, biocompatible, nontoxic, targetable and long circulating near-infrared fluorescent nanoparticulate system. It will be used for the drug delivery, controlled drug release and tumor-targeting applications, but mostly for the imaging and noninvasive therapy.

For this poly(dl-lactic-co-glycolic acid) -poly(ethylene glycol) (PLGA-PEG) nanoparticles were engineered by entrapping near-infrared fluorescent and light activated cell destruction (photosensitizing) agent, indocyanine green (ICG). Various formulations were characterized in order to achieve an optimum formulation. In-vitro release profiles of nanoparticles were determined and in-vivo blood residence time of ICG was obtained. Cell uptake and photodynamic activity of these nanoparticles were evaluated on cancer cell lines. The effect of nanoparticles on ICG distribution and pharmacokinetics assess its tumor targeting effect were characterized.

EXPERIMENTAL METHOD:

Several ratios of PLGA and PEG were chemically synthesized in our laboratory. The PLGA-PEG nanoparticles entrapping ICG were prepared by a modified spontaneous emulsification solvent diffusion method. The ICG entrapment in nanoparticles was determined and physicochemical characterization of nanoparticles were performed. The release pattern of ICG from nanoparticles was determined.

The cell uptake and photodynamic activity of the PLGA nanoparticles were evaluated on B16-F10 melanoma and C33A cervix cancer cell lines in comparison with the free drug solution. The in-vivo pharmacokinetic and biodistribution studies were performed on mice.

RESULTS AND DISCUSSION:

PLGA-PEG nanoparticles with mean diameter of about 357±21 nm and ICG entrapment of about 74% were obtained. The PLGA-PEG nanoparticles were found to have an increase in content of ICG and longer circulation time in the blood comparing to PLGA nanoparticles. The release pattern consists of an initial release phase of ICG (within 7-8 hours) followed by a relatively slow release phase.

The ICG intracellular uptake in both cell lines (B16-F10 and C33A) was concentration and time dependent through an active endocytotic transport mechanism. Once taken up by the cells ICG showed a cytoplasmic distribution and binding to the cellular proteins and structures. No effect on the cell viabilities was observed for both ICG-loaded nanoparticles and ICG solution.

After i.v. injection in mice, both ICG solution and nanoparticles formulation followed a biphasic elimination pattern from the blood with distribution in various organs such as liver, lungs, kidneys, spleen and heart. ICG removal was mainly performed by liver and had an exponential elimination profile from all organs. On the other hand, the reticuloendothelial system (liver, lungs, and spleen) was mainly responsible for the removal of nanoparticles from the blood.

CONCLUSION:

Compared with the free ICG aqueous solution, ICG-loaded nanoparticles formulation enhanced ICG stability (4-6 times), intracellular uptake (100 times), blood circulation time (2-5 times) and showed potential for photodynamic therapy. Thus, ICG-loaded PLGA nanoparticles are emerging as an ideal delivery system for ICG, exhibiting enormous potential for enhancing the efficacy of ICG for tumor diagnosis and photodynamic anticancer therapy.

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MOSTAFA SADOQI

Dr. Mostafa Sadoqi graduated from University Hassan II, Morocco with B.S. on Genie Mecanique in 1985, engineering degree on Genie Mecanique from Ecole centrale, France in 1987 and a PhD from Polytechnic University in Physics (Nanotechnology). He was an invited researcher in Biomedical Laboratory in Tsukuba, Japan from 1997 to 1999.

He is an associate professor and the founder and director of the Photonics Laboratory at the Department of Physics at St John's University since 2000.

Dr Sadoqi has published several papers on the application of laser and nanotechnology for imaging and diagnosis in scattering media such as tissue. He is a reviewer of Journal of Pharmaceutical Sciences. Also he is contributing chapters on (Vol. 2) for Wiley-VCH book series on "Nanotechnologies for life sciences" and on "colloids and nano-colloids in micro and nano-systems and biotechnologies".

WATER-SOLUBLE POLYMERS FOR CANCER THERAPY: FROM CONCEPT TO CLINIC

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The concept of targeted polymer-drug conjugates was developed to address the lack of specificity of low-molecular weight drugs for malignant cells. Features needed to design an effective conjugate include [1-3]: a polymer-drug linker that is stable during transport and able to release the drug in the lysosomal compartment of the target cell at a predetermined rate, adequate physicochemical properties of the conjugate (solubility, conformation in the biological environment), and the capability to target the diseased cell or tissue by an active (receptor-ligand) or a passive (pathophysiological) mechanism.

The advantages of polymer-bound drugs (when compared to low-molecular weight drugs) comprise [reviewed in 1-6]: a) active uptake by fluid-phase pinocytosis (non-targeted polymer-bound drug) or receptor-mediated endocytosis (targeted polymer-bound drug), b) increased active accumulation of the drug at the tumor site by targeting c) increased passive accumulation of the drug at the tumor site by the enhanced permeability and retention (EPR) effect, d) long-lasting circulation in the bloodstream, e) decreased non-specific toxicity of the conjugated drug, f) decreased immunogenicity of the targeting moiety, f) immunoprotecting and immunomobilizing activities, and g) and modulation of the cell signaling and apoptotic pathways.

Whereas the targetability of macromolecular therapeutics to cell surface antigens/receptors is well established, the manipulation of their subcellular fate is an important factor to be studied. This research direction was mainly driven by attempts to deliver genes or oligonucleotides, i.e., compounds, which may degrade in the lysosomes. However, other rationales may be important: a) The activity of many drugs depends on their subcellular location; and b) the mechanism of action of polymer-bound drug may be different than that of the free drug. Understanding these phenomena may reveal new subcellular targets specific for macromolecular therapeutics. Consequently, manipulation of the subcellular fate of macromolecular therapeutics may result in more effective conjugates.

The state-of-the-art in the development of water-soluble polymeric anticancer drugs (macromolecular therapeutics) will be demonstrated through the example of water-soluble N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer – anticancer drug conjugates. HPMA copolymer-based polymeric drug delivery systems have proved to be effective for chemotherapy, photodynamic therapy, combination therapy, and imaging. Several HPMA copolymer-based drug delivery systems have been evaluated in phase I or phase II clinical trials.

The conjugates of the future will have a double-targeting capability. They will be recognized by diseased cells and internalized by endocytic or other pathways. Once in the cytoplasm, the drug will be specifically targeted to a subcellular organelle. To achieve this, more understanding in two main areas is needed: a) Differences in the mechanism of action of free vs. polymer-bound drugs have to be determined by gene and protein expression arrays, and b) reliable technologies enabling targeting of macromolecular therapeutics to a location different from endosomes have to be developed.

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Research in the Kopecek Research Group focuses on the design, synthesis and characterization of biorecognizable biomedical polymers (home page http://www.pharmacy.utah.edu/pharmaceutics/groups/kopecek/). The main research projects relate to targetable anticancer drugs, new approaches to treat osteoporosis, colon-specific delivery systems, genetically engineered biomaterials, and self-assembling block and graft copolymers.

Dr. Kopecek received his Ph.D. in Macromolecular Chemistry and D.Sc. in Chemistry at the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czech Republic. He did postdoctoral studies at the National Research Council of Canada. In 1986 he joined the University of Utah, where he is currently Distinguished Professor of Pharmaceutics and Pharmaceutical Chemistry and Distinguished Professor of Bioengineering.

NANO-SCALE LIGAND-TARGETED DRUG DELIVERY SYSTEMS

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INTRODUCTION

Several nano-scale drug delivery systems have reached the clinic and many more are in clinical trials. These systems rely on passive targeting of the carriers to solid tumors via extravasation in areas of increased vascular permeability, including solid tumors and sites of inflammation. Ligand-targeted carriers allow improvements over passive targeting for selective delivery of drugs to disease sites. Factors that affect the binding, cytotoxicity, pharmacokinetics and therapeutic efficacy of antibody-targeted liposomes have been studied in murine xenograft models of human B lymphoma. Targeted delivery of antisense oligonucleotides (asODN) in the treatment of melanoma xenograft models and targeting of cytotoxic drugs to tumor vasculature in the treatment of neuroblastoma xenograft models have also been examined.

EXPERIMENTAL METHODS

Liposomal doxorubicin (DXR) or liposomal vincristine (VCR) was targeted via anti-CD19 or anti-CD20 monoclonal antibodies, alone or in combination. Binding, internalization and cytotoxicity were examined in vitro, and pharmacokinetics and therapeutic outcome were examined in SCID mice bearing the Namalwa human B lymphoma cell line. Results for liposomes targeted with whole monoclonal antibodies (mAb) were compared to those for liposomes targeted with Fab' fragments. In anti-vascular experiments, liposomal DXR was targeted to tumor vascular endothelial cells via an NGR-containing peptide in an orthotopically implanted model of human neuroblastoma. AsODN against the c-myc protooncogene were targeted via the anti-GD_2 monoclonal antibody to xenograft melanoma MZ2-MEL models.

RESULTS AND DISCUSSIOON

Liposomes targeted via either anti-CD19 or anti-CD20 monoclonal antibodies (mAbs) had increased binding to Namalwa cells and increased cytotoxicity relative to non-targeted liposomes. Liposomal DXR targeted via Fab' fragments had longer circulation times and better therapeutic outcome than liposomes targeted with whole mAbs. Anti-CD19-targeted liposomal VCR resulted in significant improvements in therapeutic outcome relative to anti-CD19-targeted liposomal DXR. Treatment of mice with liposomal drugs targeted via the internalizing antibody anti-CD19 resulted in increased life-spans relative to the non-internalizing antibody anti-CD20. NGR-targeted liposomal DXR bound to tumor endothelial cells, resulting in endothelial cell kill. Anti-vascular therapy led to a significant percentage of long-term survivors in this aggressive tumor model. C-myc-containing asODN resulted in increased cytotoxicity to MZ2-MEL cells compared to sense ODN or scrambled asODN. In vivo application of asODN therapy resulted in inhibition of melanoma metastases and growth control of primary, s.c., tumors.

CONCLUSIONS

In a variety of models, selected for good target access via the vasculature, ligand-targeted nano-scale drug delivery systems resulted in considerable therapeutic benefit.

ACKNOWLEGMENTS

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THERESA M. ALLEN

RESEARCH AND PROFESSIONAL EXPERIENCE:

1997 to present	Secondary appointment as Professor of Oncology, University of Alberta
1988 to present	Professor, Department of Pharmacology, University of Alberta
1981 to 1988	Associate Professor, Department of Pharmacology, University of Alberta,
1977 to 1981	Assistant Professor, Department of Pharmacology, University of Alberta,
1976 to 1977	Instructor, Department of Pathology and Department of Biochemistry; Associate Scientist, Comprehensive Cancer Center, University of Alabama, Birmingham, Alabama
1972 to 1976	Instructor, Department of Pharmacology, University of Miami, Miami, FL
1971 to 1972	Research Assistant, Department of Chemistry, McGill University

HONORS AND AWARDS: Governor General's award for highest average graduation from high school; Gold Key award for highest average B.Sc. in Biochemistry; Fisheries Research Board of Canada grant for 5 years for Ph.D. in oceanography; Defence Research Board of Canada grant for 1 year for post-doctoral research in Neurochemistry; Killam Professor, U. of Alberta, 1995-96; McCalla Research Professor, U. of Alberta, 1998-99; finalist ASTech Award for Leadership in Alberta Technology, 1999; Cygnus Award (Controlled Release Society) for excellence in guiding graduate student research; Novartis award 2000 (Pharmacological Society of Canada) for significant contributions to the advancement and extension of knowledge in Pharmacology; winner ASTech Award for Leadership in Alberta Technology, 2001; winner Alec Bangham International Award for contributions to liposome research, 2002; winner Leadership Award from the Canadian Society for Pharmaceutical Sciences, 2004.

RESEARCH: Theresa Allen is a professor of Pharmacology and an adjunct professor of Oncology at the University of Alberta. She has been active in the drug-delivery field for over 25 years and has made important contributions to the development of long-circulating liposomes, including Doxil, and to the development of ligand-targeted liposomal carriers for anticancer drugs and gene medicines. She has given over 160 invited lectures in over 25 countries.

PUBLICATIONS: over 200 peer reviewed publications and book chapters, 16 patents

POLYMER MICELLES WITH CROSS-LINKED IONIC CORES FOR DELIVERY OF ANTICANCER DRUGS

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Self-assembled block copolymer micelles have attracted great attention as nanoscale carriers for delivery of low molecular mass drugs, proteins, genes, and imaging agents. The advantages of polymer micelles for development of novel therapeutic and diagnostic modalities include the small size and core-shell architecture leading to protection of an active agent in the core by a hydrophilic polymer shell. After administration in the body the micelles circumvent renal excretion, display long circulation times, and extravasate into the disease sites with enhanced vascular permeability. Recently, nanofabrication of polymer micelles was significantly advanced by using block copolymers containing ionic and nonionic blocks (“*block ionomers*”). Such block copolymers react with oppositely charged species forming block ionomer complexes, which self-assemble into core-shell micelles. They are unique because they allow encapsulation of charged molecules into the micelle core. However, all polymer micelles have a drawback as a delivery system because they disintegrate after dilution in the body fluids, resulting in premature drug release. Herein, we report the design of novel polymer micelles with cross-linked ionic cores that display high stability.

The synthesis of these micelles involved condensation of diblock copolymers of poly(ethylene oxide) and poly(methacrylic acid) (PEO-*b*-PMA) by divalent metal cations into spherical micelles of core-shell morphology. The core of the micelle was cross-linked further through the use of bifunctional agents, and cations were removed by dialysis (Figure 1). As a result of cross-linking reaction, narrowly distributed particles of ca. 170 nm diameter with a net negative charge (zeta-potential, ξ = -19 mV) were formed. The particles were stable and revealed no size change even upon a 100-fold dilution. No aggregation or precipitation was observed for months. They can be freeze-dried and reconstituted in aqueous dispersion practically without change in their initial size. The resulting micelles had a core-shell structure, as was confirmed using ^{1}H-NMR spectroscopy. The core comprises a network of the cross-linked polyanions, which is surrounded by the shell of hydrophilic PEO chains. As expected, cross-linking of the core domain assisted in the maintenance of their spherical morphology. Furthermore, these micelles displayed the pH- and ionic strength-responsive hydrogel-like behavior due to the effect of the cross-linked ionic core. Such behavior may be instrumental for the design of drug carriers with controlled loading and release characteristics.

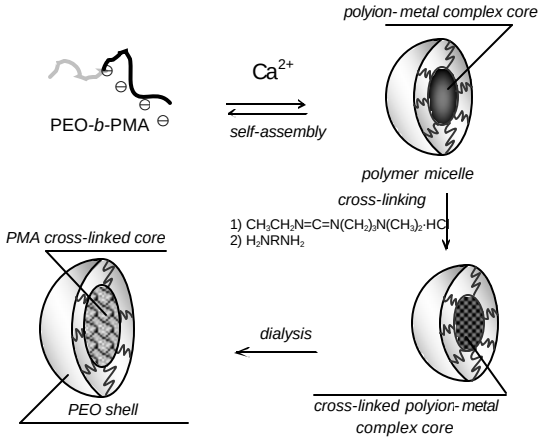

Figure 1. Synthesis of polymer micelles with cross-linked ionic cores

Cisplatin, a potent anticancer drug, was immobilized in the cross-linked polymer micelles by simple mixing with aqueous dispersion of polymer micelles. The drug-loaded micelles were stable in aqueous dispersions exhibiting no aggregation or precipitation for a prolonged period of time. Slow release of platinum complexes was observed in a sustained manner from the drug-loaded micelles in physiological saline. *In vitro* studies using human A2780 ovarian carcinoma cells demonstrated that the cross-linked micelles rapidly internalized and delivered cisplatin into cells. Cross-linked micelles alone were not toxic at concentrations used for the treatment by cisplatin/polymer micelle formulation, while cisplatin incorporated in the micelles displayed lower cytotoxic activity than free cisplatin. Since the Pt complexes released from the micelles play an essential role in the cytotoxic activity, this range in reduction in cytotoxicity may be consistent with the sustained manner of Pt release.

Acknowledgement: We acknowledge the support of the National Science Foundation (DMR 0071682).

TATIANA BRONICH

Tatiana Bronich is a Research Associate Professor at College of Pharmacy and Center for Drug Delivery and Nanomedicine at University of Nebraska Medical Center. Her educational background includes undergraduate training in Chemistry at Moscow State University (M.S., diploma with distinction), Russia, followed by graduate studies at the Department of Polymer Sciences of the same University. Dr. Bronich's research interests are in the area of self-assembling polymer materials and applications of these materials in medicine. Of special interest is the design and study of novel types of functional materials based on complexes formed between block ionomers and oppositely charged polymers and low molecular weight amphiphilic molecules. These systems are of great fundamental importance as models of biological systems formed as a result of self-assembly processes. In addition, her recent work has expanded to include the application of these amphiphilic block copolymers and block ionomer complexes in drug delivery to treat cancer and the development of the polycation-DNA complexes for gene delivery.

TARGETING OF CANCER DRUGS TO SOLID TUMORS BY THERMALLY RESPONSIVE POLYMER CARRIERS

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This talk will describe thermal targeting of cancer therapeutics to solid tumors by two different classes of thermally responsive recombinant elastin-like polypeptides (ELPs) that exhibit a lower critical solution temperature transition slightly above 37 °C. The first generation of ELPs that we have designed as thermally triggered molecular actuators for drug delivery are pseudorandom copolymers of the VPGXG repeat where the mole fraction of X and the polymer chain length were precisely specified so the polypeptide would undergo its phase transition between 37 and 42 ºC. In vivo fluorescence videomicroscopy of human tumors implanted in nude mice demonstrated that the phase transition of this thermally responsive ELP occurs in heated tumors resulting in the formation of micron-size aggregates of the thermally responsive ELP within the heated tumor. The phase transition results in a ~two-fold increase in tumor localization compared to the same polypeptide without hyperthermia even for heating periods as short as one hour. We have observed that thermally cycling the tumor can further increase the uptake of the ELP within the tumor by five-fold compared to the same polypeptide without hyperthermia. Doxorubicin was conjugated to this first generation ELP carrier via an acid labile hydrazone bond to enable release of the drug in the acidic environment of lysosomes. The ELP-doxorubicin conjugate was endocytosed by squamous cell carcinoma cells and trafficked into lysosomes, as observed by the colocalization of the doxorubicin with a lysosome-specific dye by confocal fluorescence microscopy. The ELP-doxorubicin conjugate and free drug exhibited equivalent cytotoxicity in cell culture. These results suggest that thermal targeting of a soluble macromolecular carrier may be useful for the delivery of cancer therapeutics.

A second generation of diblock ELPs will also be described that function as temperature triggered polymer amphiphiles. Two classes of ELP amphiphiles have been synthesized: the first class form monodisperse, ~60 nm diameter micelles in the range of 37-42 ºC, a range approved for clinical hyperthermia of solid tumors, which will allow the multivalent presentation of tumor specific ligands only in tumors, thereby enhancing their accumulation in tumors. The second class of diblock ELPs are designed to undergo their monomer to micelle transition at room temperature to enable thermally triggered loading of drugs or imaging agents into the core of the micelle, followed by release of their contents upon undergoing their micelle- aggregate transition in heated tumors.

ASHUTOSH CHILKOTI

Ashutosh Chilkoti received his B. Tech. in Chemical Engineering from the Indian institute of Technology, Delhi in 1985, a Ph.D. in Chemical Engineering from the University of Washington in 1991, and was a post-doctoral fellow in the Department of Bioengineering at the University of Washington from 1992 to 1995. He was appointed as an Asst. Prof. of Biomedical Engineering at Duke University in 1996, and promoted to Assoc. Professor in 2002. In 1998, Prof. Chilkoti won the CAREER award from the NSF, in 2002 the 3M non-tenured faculty award, and in 2003 and in 2005 was awarded the Distinguished Research Award from the Pratt School of Engineering at Duke University. He was appointed Associate Director of the Center for Biologically Inspired Materials and Materials Systems at Duke University in 2002. His areas of research include Biomolecular Engineering with a focus on stimulus responsive biopolymers for protein purification and drug delivery, advanced coatings for control of protein and cell adhesion for biosensors and biomaterials, and development of new nano-microscale bioanalytical technology. He has co-authored over 90 publications, and has 10 patents awarded or in process, and serves on the Scientific Advisory Board of 2 companies and serves on the Editorial Board of Biomolecular Engineering and of Protein Engineering, Design and Selection.

VIP-RECEPTOR TARGETED PHOSPHOLIPID NANOCARRIERS FOR IN VIVO ANTI-CANCER DRUG DELIVERY

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INTRODUCTION

Efficacy of chemotherapy is hampered by serious dose limiting side effects, due primarily to non-specificity of the anti-cancer drug. Recently, we have developed biocompatible and biodegradable nanocarriers to target the cells that overexpress vasoactive intestinal peptide (VIP) receptors (VIP-R) during cancer or inflammation development. The nanocarriers are sterically stabilized micelles (SSM) and sterically stabilized mixed micelles (SSMM) composed of amphiphilic PEGylated phospholipid, disteraroyl phosphatidylethanolamine conjugated to polyethylene glycol 2000 (DSPE-PEG_{2000}) or DSPE-PEG_{2000}:phosphatidylcholine (90:10), respectively. Therefore, these micelles are long circulating in vivo. Their surfaces are further modified with VIP in order to target the carriers to diseased cells. Water-insoluble anti-cancer or disease modifying drug (DMD) molecules can be solubilized in the hydrophobic cores of the micelles and specifically delivered to the cancer or inflamed tissues due to interaction with VIP-R on the cells, after extravasation of the nanocarrier through the leaky vasculature. In this study, we tested our hypothesis that anti-cancer and DMD molecules solubilized in micelles with actively targeting VIP will exhibit higher efficacy and lower toxicity than free drug. We anticipate that the drug carrier being in the nano-size range, should pass selectively through the leaky vasculature of diseased organs, but they are too big to extravasate to the normal healthy tissues and cause toxicity. Since the nanocarrier evades uptake by mononuclear phagocytic system and VIP-R is expressed predominantly in the extravascular space, the efficiency of active targeting would be high without concomitant VIP toxicity.

EXPERIMENTAL METHODS

We tested paclitaxel (P)-SSMM-VIP in a carcinogen-induced breast cancer rat model and camptothecin (C)-SSM-VIP in a collagen-induced arthritis (CIA) mouse model. Carcinogen-induced breast cancer in rats (1) and CIA in mice (2) were developed as described previously. Paclitaxel and camptothecin were solubilized in SSMM and SSM, respectively by co-precipitation/reconstitution. Lipid-VIP conjugate prepared as optimized previously (1), was incubated with paclitaxel and camptothecin micelles to form actively targeting drug-loaded micelles with VIP on their surfaces. Efficacy, pharmacokinetics and biodistribution, as well as safety of paclitaxel-SSMM-VIP and camptothecin-SSM-VIP, in comparison to Taxol® and camptothecin alone, were evaluated in vivo.

RESULTS AND DISCUSSION

More than 3-fold increase and 2-fold increase were achieved in paclitaxel distribution to tumor tissues with P-SSMM-VIP when compared to Taxol® and P-SSMM, respectively. Furthermore, distribution of paclitaxel to systemic tissues associated with toxicities, such as the bone marrow and spleen, was also significantly reduced for P-SSMM-VIP in comparison to both P-SSMM and Taxol®. Treating tumor-bearing rats with a low dose of paclitaxel (1mg/kg) resulted in 80% tumor regression in 30 days with P-SSMM-VIP, whereas P-SSMM and Taxol® demonstrated only 40% and 25% tumor regression, respectively. Treating CIA mice with CPT-SSM-VIP also showed a 10-fold and 3-fold activity after 30 days when compared to CPT alone and CPT-SSM, respectively. Hence, drug-loaded nanocarriers with VIP actively targeting the target cells at cancer or inflammation sites reduced the effective drug dose and adverse effects significantly.

CONCLUSIONS

In conclusion, we suggest that surface modification of drug-loaded PEGylated phospholipid micelles with VIP for active targeting is successful in increasing therapeutic effectiveness in cancer chemotherapy and rheumatoid arthritis with lower drug toxicity. We propose P-SSMM-VIP and C-SSM-VIP as anti-cancer and DMD nanomedicines of the future.

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ACKNOWLEDGEMENTS

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She is a member of several professional organizations including the American Academy of Nanomedicine. She has received several awards including New Investigator Award from AACP, UIC Inventor of the Year 2003, and UIC Woman of the Year 2003. She is on the Editorial Board of journal of Nanomedicine: Nanotechnology, Biology and Medicine. She is also a member of Nanotechnology and Nanoscience Special Emphasis NIH Grant review panel.

NANOMEDICINE: A EUROPEAN (PERSONAL) PERSPECTIVE

R. Duncan

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Statistics tell us that someone dies of cancer every 4 seconds, of AIDS every 11 seconds and of Alzheimer's disease every 86 seconds. The average UK pharmacy has about four patients with multiple sclerosis. The pressing need for improved therapies for these and other life-threatening, debilitating and chemotherapy-resistant diseases is obvious. There is increasing anticipation that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. This has prompted many governmental and funding agencies to strategically review the field[1], and the primary objectives have been to ascertain current status, to establish a common terminology, to assess potential benefits and risks and to establish priorities for future funding initiatives. When a field suddenly becomes fashionable, it is important to keep perspective and, most importantly, distinguish the science fact from science fiction. Although not widely appreciated, progress in the development of nano-sized hybrid therapeutics and nanosized drug delivery systems over the past decade has been remarkable. A growing number of products including liposomes[2], antibodies and their conjugates[3], nanoparticles[4] and polymer therapeutics[5,] have already secured regulatory authority approval. In turn, these products are supported by a healthy clinical development pipeline, and they can rightly be viewed as the first "nanomedicines" and they are already bringing clinical benefit to thousands of patients.

Even agreement on the basic definitions is proving a challenge to those working in the multidisciplinary field of nanotechnology[6]. Within the confines of the nanoscale size range, the discipline of "nanomedicine" can be best defined as the science and technology of diagnosing, treating and preventing disease. On one hand, device miniaturization is providing exciting opportunities, whilst on the other progress in synthetic and supramolecular chemistry is generating ever more sophisticated, multi-component nanosized technologies. Recently the European Science Foundation's Forward Look on Nanomedicine identified five distinct, but overlapping sub-themes contributing to the field of nanomedicine. These are (i) analytical techniques and diagnostic tools, (ii) nano-imaging and manipulations, (iii) nanomaterials and nanodevices, (iv) nanomedicines designed either as biologically active therapeutics or drug delivery systems, and (v) all the issues relating to their pharmaceutical development, and clinical use with particular regard to potential toxicity. Each of these topics is equally important and must be supported by multi-disciplinary research teams in academia and industry if the full potential is to be realized. Will nano-robots be routinely used to diagnose, locate and then successfully treat disease, as and when needed? Why not? The notion may seem fantastic, and there are many technical and safety issues to address, but it is noteworthy that many of the drug delivery technologies in everyday use today were dismissed as impractical 30 years ago, being then viewed as science fiction.

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RUTH DUNCAN

Ruth Duncan is Professor of Cell Biology and Drug Delivery at the Welsh School of Pharmacy Cardiff University where she directs the Centre for Polymer Therapeutics. She established the UK Cancer Research Campaign's Polymer Controlled Drug Delivery Group at Keele University, UK in the 1980's before joining Pharmacia, Milan as Head of New Technologies. On returning to academia she established the Centre for Polymer Therapeutics at the London School of Pharmacy and relocated to Cardiff in 2000. Throughout she has maintained an interest in the rational for design of polymer therapeutics suitable for transfer into clinical testing. She has produced more than 250 articles, reviews and patents, and her work has been recognised via awards including the Pfizer Prize, Hlasek Medal of the Czech Academy of Sciences, Controlled Release Society Young Investigator Award, The Royal Society for Chemistry Interdisciplinary Award and the Berlin-Brandenburg Academy of Sciences: Monika-Kutzner Prize for Innovation in Cancer Research. She is also an elected member of the Mainz Academy for Science and Literature and in 2004 was Chair of the Steering Committee responsible for the European Science Foundation's Forward Look on Nanomedicine.

THE STATUS OF NANOMEDICINE RESEARCH IN JAPAN

K. Kataoka

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As seen in Figure 1, nanobiotechnology research initiative has been organized under the strong cooperative action of related ministries and governmental agencies in Japan, such as Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Ministry of Health, Labor an Welfare (MHW), Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of Economy, Trade, and Industry (METI), and Ministry of Environment. This initiative includes both fundamental and applied research area in nanobiotechnology, and the most focused area from the standpoint of social security, health and welfare, and economy is "Nanomedicine", including DDS, gene delivery, tailor-made diagnosis/therapy, nano-medical devices, and tissue engineering.

Notably, DDS and gene delivery has been recognized as the key technology in the field of "Cell therapy", which has recently been decided by Nanotechnology Committee of MEXT as the guiding concept in nanomedicine research. "Cell therapy" is the general term given for the methodology of nano therapy at the cellular level by infusion/transplanting human cells as well as selective delivery of genes and drugs into the target cells. This presentation will overview the "Cell therapy" research program in Japan to discuss the future perspective of DDS and gene delivery research in the field of nanomedicine.

KAZUNORI KATAOKA

Kazunori Kataoka, Ph.D., is a Professor of Biomaterials at Graduate School of Engineering, the University of Tokyo, Japan. He has been appointed joint position since 2004 from Graduate School of Medicine, the University of Tokyo as a Professor of Clinical Biotechnology at Center of Disease Biology and Integrative Medicine.

Dr. Kataoka received a B.S. from the University of Tokyo in 1974 and a Ph.D. from the same University in 1979. He served as a Research Associate and an Associate Professor at the Institute of Biomedical Engineering, Tokyo Women's Medical College, from 1979 to 1989. In 1989 he assumed Associate Professor position at Science University of Tokyo. He became full Professor there in 1994. In April, 1998, he moved to the University of Tokyo as a Professor of Biomaterials.

Dr. Kataoka is an Associate Editor of the Journal of Biomaterials Science, Polymer Edition, Journal of Controlled Release (Controlled Release Society), and Biomacromolecuels (American Chemical Society), and an Executive Editor of Advanced Drug Delivery Reviews, on the Editorial Board of Journal of Drug Targeting, and Materials and Engineering. He held visiting professorship at the University of Paris XIII in 1992, 1996, and also in 2000. He received the Society Award from the Japanese Society for Biomaterials in 1993. In 1995, he received 1994 Journal of Controlled Release, Jeorge Heller Outstanding Paper Award from Controlled Release

Society. Also from Controlled Release Society, he received 1996 Outstanding Pharmaceutical Paper Award. He has been a Fellow of the American Institute of Medical and Biological Engineering since 1999 and a Fellow of Biomaterials Science and Engineering since 2004. He received Society Award from Society of Polymer Science, Japan in year 2000, and in the year 2005, received Clemson Award for Basic Research from Society for Biomaterials, USA.

Dr. Kataoka is the author of more than 300 scientific papers. His current major research interest includes the molecular design of cellular specific polymers and the development of new polymeric carrier systems, especially block copolymer micelles, for drug and gene targeting.

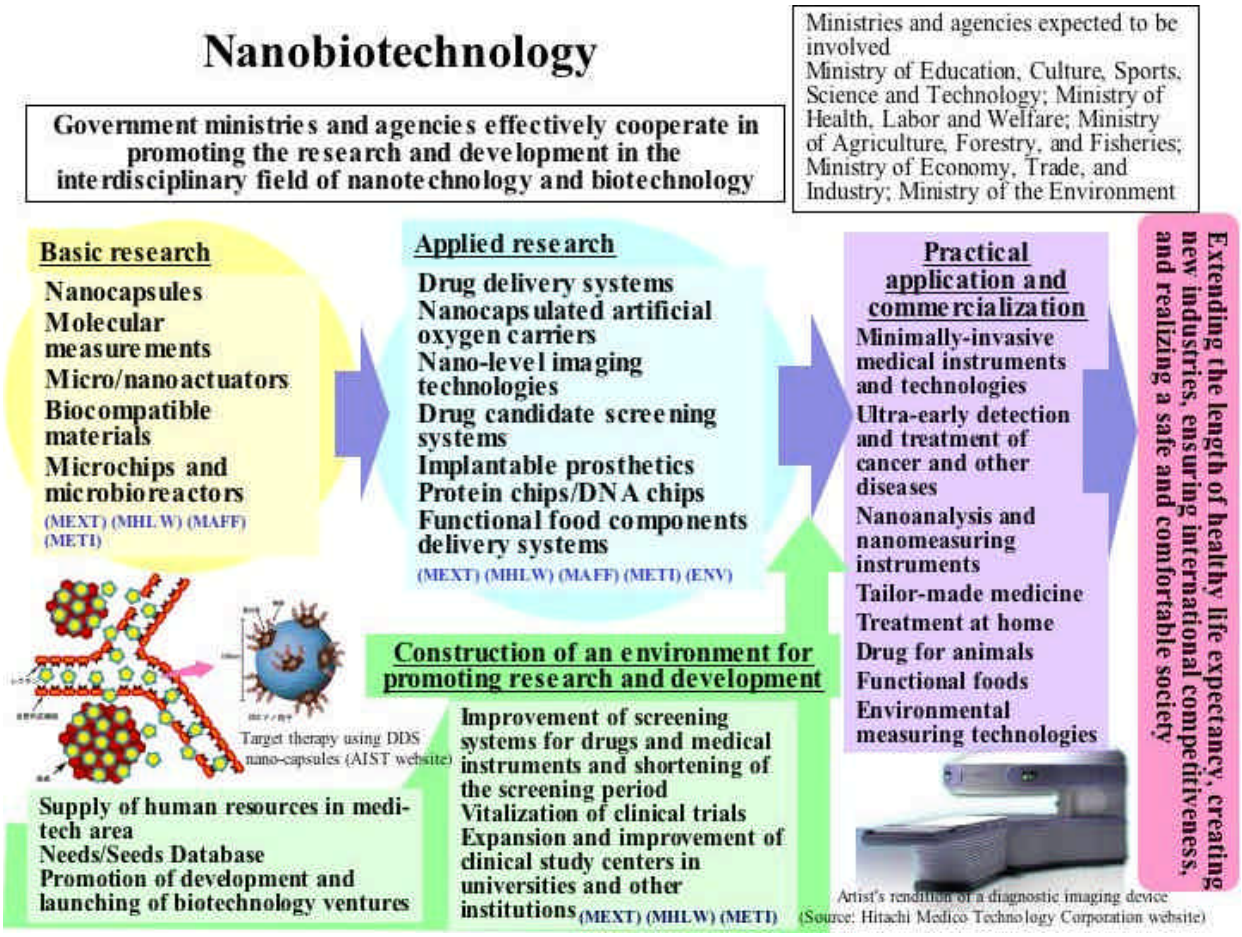

Fig. 1 Nanobiotechnology research initiative in Japan

NANOMEDICINE: THE US PROSPECTIVE

A. V. Kabanov

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Nanomedicine develops nanomaterials and devices operating at the nanoscale to diagnose, treat and monitor diseases. It is expected that nanomedicine would yield implantable devices, 100,000 times smaller than the head of a pin, which will effectively detect diseases without surgical invasion and then, will eradicate the diseased cells by precisely "pumping" medicine to them. The nanomedicine field joins engineering science with pharmaceutical and medical sciences to translate advances in nanotechnology research into clinical practice. A major science and technology effort is underway in the United States to address this goal. This effort involves the Government, the Industry and the Scientific Community. The U.S.A. National Nanotechnology Initiative (NNI) is a federal R&D program established to coordinate the multi-agency efforts in nanoscale science, engineering, and technology (http://www.nano.gov/). The National Institutes of Health (NIH) has recently launched several major initiatives including the National Cancer Institute's Nanotechnology Alliance in Cancer (http://nano.cancer.gov/index.asp) and the NIH Nanomedicine Roadmap Initiative (http://nihroadmap.nih.gov/nanomedicine/). Several scientific organizations in the United States including the recently founded American Academy of Nanomedicine (http://www.nanomedacademy.org/) have focused on the nanomedicine research. While small and middle size drug delivery companies have developed nanotechnology based modalities for delivery of drugs a recent study published by Lux Research suggests that major pharmaceutical companies globally are committing relatively little money or people to nanotechnology research. This may, however, soon change as nanomedicine may provide major competitive advantages to industry by changing the way how therapies are administered. Several nanomaterial-based therapies have already been approved for clinical use and many more nanomaterials are evaluated in clinics. Thus nanomedicine is not only "a futuristic" but is also "a realistic" field with the near-term prospective to improve human health.

ALEXANDER KABANOV

Alexander Kabanov, born in Moscow, USSR in 1962, graduated with M.S. degree from M.V. Lomonosov Moscow State University (MSU) in 1984, obtained Ph.D. (1987) and D.Sc. (1990). Held academic positions in the School of Chemistry, MSU from 1987 to 1997, was appointed an adjunct professor in 2002. Since 1994, has worked at the College of Pharmacy, University of Nebraska Medical Center, where currently is a Parke-Davis Professor and Director, Center for Drug Delivery and Nanomedicine. Co-founded Supratek Pharma Inc. in 1994. One of the founders and the first director of the University of Nebraska Center for Drug Delivery and Nanomedicine (2004). Made substantial contributions in the fields of micellar enzymology, nanomedicine, drug and gene delivery. Was one of the first to use synthetic polycations and polymeric micelles for DNA and drug delivery (1989), to develop environmentally responsive nanomaterials using block iomomer complexes (1995) and discovered the effects of synthetic polymers on genomic responses in the body – "polymer genomics" (2002). Has published over 150 scientific papers and awarded over 100 patents worldwide. Awards include the Lenin's Komsomol Prize (1988) and the NSF Career Award (1995).

NANOPARTICLES – THE REGULATORY PERSPECTIVE

M. A. Khan

Division of Product Quality Research, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD-20993-002, USA

Nanoparticles as drug delivery systems include particles that are in nanometer size range. In the past nanoparticles have been obtained by a variety of "top-down" approaches including grinding, nanoprecipiation, nanoencapsulation, and nanocrystallization. By these approaches, coarser particles have been size-reduced to nanometer size range for benefits that include increased surface area/increased reactivity, enhanced solubility/increased rate of dissolution, increased oral availability, more rapid onset of therapeutic action, and/or reduced dose.

The current symposium as well several others in the recent past are clearly showing a trend of the availability of novel nanoparticles that are prepared by the assembly or manipulation of atomic scale chemicals into nano-sized particles as drug products. This approach is opposite to that of the top-down approach. Particles prepared by these techniques (e.g. Fullerenes, Dendrimers) appear to be used for targeting to specific sites in the body. This symposium has shown its utility in drug targeting to tumor as well as imaging for diagnostic purposes.

The US FDA has approved several complex dosage forms as INDS, NDA, and ANDAS. When a product is well understood, well characterized, with experimental evidence of safety and efficacy, FDA has the means, resources and authority to approve such products for the benefit of public.

A well-characterized product would show predictability of physico-chemical and biological properties, freedom from several types of toxicities, acceptable stability, bioavailability, and efficacy. To address these issues with nanoparticles, the FDA has proactively formed a nanotech working group that is working collaboratively with research and academic institutions, NIST, NIH, and several other centers within the FDA. As more knowledge becomes available, it is likely that the manufacturers of nanotech product will demonstrate the safety and efficacy with modern tools and techniques. Once the preclinical safety and probable/possible effect of nanoparticles in human drug use is determined, the investigators apply for the investigational new drug (IND). The IND authorizes the investigators to start the Phase I clinical studies for safety of nanoparticles in humans.

MANSOOR A. KHAN

Dr. Mansoor A. Khan is the Director of Product Quality Research at FDA. Prior to joining FDA, Dr. Khan was a Professor of Pharmaceutics and Director of Graduate Program in the School of Pharmacy at Texas Tech University Health Sciences Center. He also served as Assistant and then Associate Professor at the Northeast Louisiana University School of Pharmacy. He is a registered pharmacist and has earned his Ph.D. degree in Industrial Pharmacy from the St. John's University School of Pharmacy at New York in 1992. He has published over 110 peer-reviewed manuscripts, four texts including the "Pharmaceutical and Clinical Calculations," three book chapters, and more than 75 presentations in various meetings. Dr. Khan graduated 10 Ph.D.s, who are all employed by leading pharmaceutical industries in the US. Dr. Khan's research focus is primarily in the area of controlled drug delivery of challenging molecules. He examines the critical process and formulation variables of solid oral dosage forms. His most recent grants and publications are in the area of oral delivery of macromolecules, nanoparticles, and aqueous-based coating dispersions. He has held several leadership positions at the AAPS, and was recently elected as the Vice-Chair of PDD section. He also serves on the editorial board of Pharmaceutical Technology, the journal of Clinical Research and Regulatory Affairs, and the journal “Critical Reviews and Therapeutic Drug Carrier Systems”.

Poster Abstracts

IN VITRO EVALUATION CONTROLLED RELEASE STUDY FOR METFORMIN HYDROCHLORIDE POLYMERIC HYDROGEL MATRICES

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The hydroxyethylcellulose hydrogels were prepared by its chemical crosslinking using glyceraldehyde, and tetramethylolurea resin were prepared and polymerized by hydrochloric acid. Several tablets were prepared as polymeric matrix and polymeric reservoir systems from hydroxyethylcellulose hydrogels, loaded with Metformin hydrochloride in ratio (25%, 34%, 50% wt/wt).The two polymeric matrix systems were prepared by compression molding and dip coating process respectively.

The release rate of Metformin hydrochloride from polymeric matrix devices was studied by using u.v. technique at constant temperature (37°C). The effect of pH on the release rate of Metformin hydrochloride in (PBS, pH=7.4), SGF, and SIF was studied. The swelling behavior of some prepared samples was also studied and the result showed greater increase in swelling ratio at lower pH values.

DEVELOPMENT OF A NOVEL NANO-VESICLE FOR THE TREATMENT OF DISEASES WITH INFLAMMATORY COMPONENT

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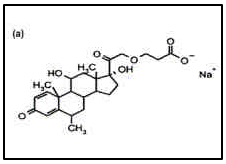
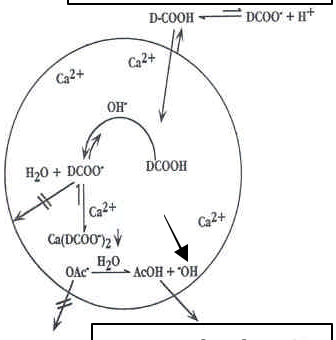
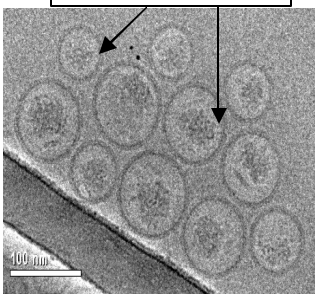


Glucocorticosteroids are the drugs of choice in many diseases with inflammatory components. However, due to unfavorable pharmacokinetics and/or unfavorable biodistribution in many such situations the efficacy of these drugs is not good enough and the drugs are highly toxic. We developed a liposomal formulation based on (≤ 100 nm) sterically stabilized liposomes containing the weak amphipathic acid methylprednisolone-succinate (MPS). The drug was remote-loaded into the liposome aqueous phase using a calcium acetate gradient. We demonstrated the therapeutic efficacy of this formulation on animal models (mice, rats) having adjuvant induced arthritis and experimental autoimmune encephalomyelitis.

Mechanism of loading



Encapsulated MPS



DEVELOPMENT OF ANTI-INFLAMMATORY NANO-VESICLE

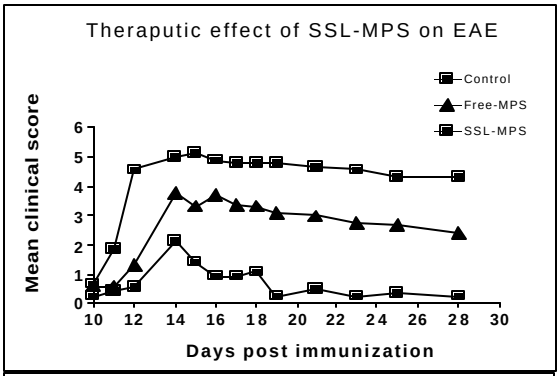
Our drug of choice was methylprednisolone succinate, this is amphipathic weak acid and therefore it can be remote-loaded into liposomes via the calcium acetate gradient.

Sterically-stabilized liposomes (≤ 100 nm) composed of hydrogenated soy phosphatidylcholine/cholesterol/ (mPEG-DSPE) (PEG = 2000 Da) 55/40/5 (mole ratio) were prepared. using a calcium acetate gradient under conditions of [Ca acetate] liposome >> [Ca acetate] medium. Such a gradient is characterized by an excess of the impermeable Ca2+ ions over the permeable (as acetic acid) of the acetate, thereby leading to a pH gradient [pH] liposome > [pH] medium. Such liposomes when incubated with MPS pumped into their alkaline aqueous phase form a calcium salt which precipitates inside the liposomes, thereby increasing the level of stable loading. The loading process is continuous as long as there is excess of intraliposomal acetate. The loading can be defined as the exchange of acetate with MPS. Such loading also enables drug release in the tissue at a rate sufficient to achieve therapeutic efficacy

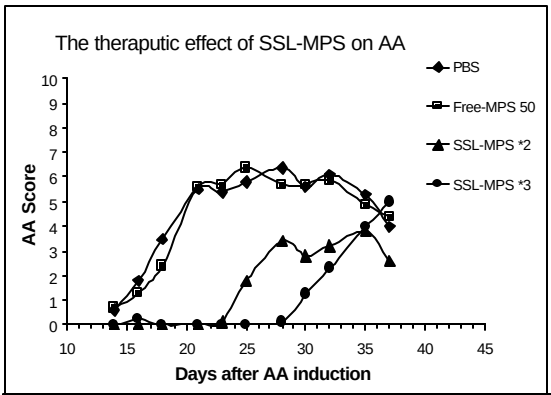
THERAPEUTIC SUCCESS

Animal models clearly show the superiority of the SSL-MPS compared to administering the "free drug". We studied two in-vivo models to simulate pathological conditions in humans that are being treated with methylprednisolone sodium succinate:

1. Multiple sclerosis (experimental autoimmune encephalomyelitis, EAE)
2. Adjuvant-induced arthritis (AA)



6-7 weeks old SJL mice were vaccinated to induce EAE and at day 10 after immunization mice were treated with 50mg/kg BW SSL-MPS (days 10, 14, 18) , free-MPS 50mg/kg BW (days 10,14,18) and dextrose 5% (days 10,14,18)



Lewis rats were vaccinated to induce AA and treated on days 10 and 14 with i.v. injections of free-MPS (10 50 mg/Kg bw), SSL-MPS (10, mg/Kg bw) or with PBS (control). An additional group of rats recieved a third i.v. injection of SSL-MPS (10 mg/Kg bw) on day 18

TREATING HEAD AND NECK CANCER WITH TARGETED POLYMERIC CONJUGATES

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INTRODUTION

Head and neck cancer is the sixth most common cancer in European countries as well as in the United States. Squamous-cell carcinoma (HNSCC) counts for more than 90% of all causes [1]. Despite advances in surgical and other treatments, survival rates are not improving because of local recurrences. The efficiency of all therapeutic modalities available at the moment is less than 50% in five year follow-up. The necessity of finding more successful approaches to the treatment is evident.

Water-soluble synthetic polymers based on an *N*-(2-hydroxypropyl) methacrylamide (HPMA) backbone are recognized as useful carriers in targeted drug delivery. Targeted conjugates, or prodrugs, containing a cytotoxic drug bound to a polymeric backbone targeted with a monoclonal antibody represent a promising novel modality in cancer management [2]. Preclinical studies proved that these therapeutics posses not only cytostatic but also immunoprotecting activities and, at the same time, show only minimal side-effects [3]. The polymeric prodrugs were considerably efficient even in experimental models of cancer which were poorly curable with available chemotherapy [4]. One particular malignancy with rather bad prognosis even when treated with chemotherapeutics is head and neck squamous cell carcinoma (HNSCC).

EXPERIMENTAL METODS

The cytotoxic activity of samples was tested by their antiproliferative capacity determined as [^{3}H]thymidine incorporation into the cell DNA and evaluated as IC_{50} (concentration of doxorubicin which inhibits proliferation of treated cells to 50% of the control cell level). The binding capacity was tested by FACS and expressed them as mean fluorescence intensity (MFI) [5].

RESULT AND DISCUSSION

It was demonstrated that HNSCC in general and FaDu experimental cell line in particular is highly overexpressing EGFR. Transferrin receptor (CD71) is a marker of activated and dividing cells and it is also strongly expressed on these cancer cells. Therefore we designed an HPMA conjugates containing doxorubicin targeted with anti-EGFR mAb or with anti-CD71 mAb and compared the efficiency to non-targeted conjugate and with free anti-EGFR mAb. We proved that *in vitro* cytotostatic effect of the anti-EGFR targeted conjugated evaluated as IC_{50} is approximately twenty fold higher compared to non-targeted conjugate and ten fold higher compared to free mAb and anti-CD71 targeted conjugates.

CONCLUSION

Targeted conjugates are in vitro much more cytostatic than non-targeted conjugates or free monoclonal antibody. Especially anti-EGFR targeted conjugates seem to be a perspective modality in treating HNSCC.

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ANIMAL MODELS FOR THE EVALUATION OF BIODISTRIBUTION AND EFFICACY OF POLYMER THERAPEUTICS TARGETING SOLID TUMORS

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INTRODUCTION: Lack of specificity is a major problem of the currently available anticancer agents. Advances in cancer therapy through tumor targeting will depend on exploiting critical differences between malignant and normal tissues. The identification of specific genetic changes in tumors is one, that of a distinctive tumor physiology another possible approach. Tumor blood vessels are unique and characterized by leakiness caused through enhanced neovascularization [1-2]. Therefore, therapies aiming to inhibit blood vessel formation or to exploit the enhanced permeability and retention effect (EPR) of tumor vasculature seem promising targeting concepts [1-2]. The latter include nanoparticles and polymers. To facilitate the preclinical and clinical development of polymer therapeutics and nanoparticles for diagnosis and treatment of solid tumors, we designed *in vivo* models that can be used to study efficacy and biodistribution.

EXPERIMENTAL METHODS: Engraftments of human tumor cell lines and tissues in nude mice have proven valuable systems in cancer drug development [3]. In particular, subcutaneously (s.c.) growing human tumor xenografts derived from primary tissues mimic closely the histology and characteristics of the original patient tumor despite the fact that stroma and blood supply are provided by the mouse. E.g. production of growth factors by the human tumor cells can stimulate angiogenesis and neovascularization [4]. In this study we determined vascular permeability (VP), vessel density (VD) and VEGF expression in 37 human tumor xenograft models by Evans blue extravasation, Hoechst H33342 staining of tumor blood vessels and by ELISA assay, respectively. Variables were tumor size and tumor histology. The latter include bladder (2), colon (4), gastric (2), head and neck (1), lung (5), breast (8), melanoma (2), ovarian (2), pancreas (2), prostate (2), renal (5) and soft tissue (2) tumors.

RESULTS: Uptake of Evans blue bound to serum albumin by leaky tumor blood vessels was examined and showed intra- and inter-tumoral heterogeneity. Dye retention was markedly higher in renal cell carcinomas compared to cancers of other histology and was dependent on tumor size. An optimal uptake of macromolecular Evans blue was seen in well vascularized tumors at a mean volume of 300-500 mm^3. Smaller and larger tumors (<300 and >500 mm^3) had a reduced retention. The highest EPR effect at 500 mm^3 was found in a renal cell cancer model, RXF 1220, which retained an average of 216 µg/g (8.7% dose/g) tumor, whereas an ovarian tumor, OVXF 1023, exhibited the lowest VP (5.0 µg/g or 0.2% dose/g tumor). The extent of EPR was paralleled by the expression of vascular permeability factor VPF/VEGF and by microvessel density.

CONCLUSIONS: Our data demonstrate that solid human tumors differ in their expression of factors determining angiogenesis and hence EPR. Whilst VP, expression of VEGF and VD correlate mostly well in a certain human tumor xenograft model, they can be very variable within and between tumor types although tumor size and passage numbers are similar. This reflects the situation found in primary patient tumors. Due to such differences, it will be critical to select appropriate, stable *in vivo* models for the evaluation of compounds which target tumor vasculature and angiogenesis and to include imaging methods for the selection and monitoring of patients who might receive these therapies. Some tumor types, e.g. prostate and renal cell cancers seem more suitable to study tumoritropism and efficacy of nanoparticles and polymer therapeutics.

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PLGA NANOPARTICLE-APTAMER BIOCONJUGATES AS DRUG DELIVERY VEHICLES FOR TARGETED PROSTATE CANCER THERAPY

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INTRODUCTION

Aptamers have emerged as a new class of nucleic acid ligands that rival antibodies in their potential applications in therapeutics and diagnostics. When compared to antibodies, aptamers have many favorable characteristics, such as small size, low immunogenicity, and easy preparation.[1] We have designed and synthesized bioconjugates comprised of controlled release polymer (PLA-PEG-COOH) nanoparticles and aptamers which bind to the Prostate Specific Membrane Antigen (PSMA), and demonstrated that these bioconjugates can efficiently target and get taken up by LNCaP cells, a PCa cell expressing the PSMA protein. We observed an increase of cell binding and internalization by nearly two orders of magnitude as compared to PC3 cells that do not express the PSMA protein.[2] In our current study we encapsulated docetaxel into PLGA-PEG-COOH/aptamer bioconjugates and evaluated their antitumor effect in LNCaP tumor bearing nude mice.

EXPERIMENTAL METHODS

Docetaxel encapsulated polymeric nanoparticle-aptamer bioconjugates (Dtxl-NP-Apt) were prepared through nanoprecipitation of an acetonitrile solution of docetaxel and PLGA-PEG-COOH into water, followed by the conjugation of nanoparticles with amine-terminated PSMA aptamer. PSMA-expressing xenograft tumors were induced in the flank of balb/c nude mice by subcutaneous injection of LNCaP cells. After 3 weeks, Dtxl-NP-Apt, Dtxl-NP and Dtxl were injected to corresponding groups intra-tumorally at 40mg Dtxl/kg.

RESULTS AND DISCUSSION

The mice were administered at their maximum tolerable dose (40 mg Dtxl/kg). The maximum weight loss of Dtxl-NP-Apt group was 8.2%, comparing to Dtxl-NP and Dtxl group that had maximum body weight loss of 19.9% and 16%, respectively. On day 41, tumors in five out of seven animals were totally reduced in the Dtxl-NP-Apt group, while only two complete tumor regressions were observed the Dtxl-NP group on day 44. The Dtxl and no-treatment groups did not experience any total tumor regressions. The aptamer-conjugated NPs were significantly better than the controls in terms of both toxicity and antitumor efficacy.

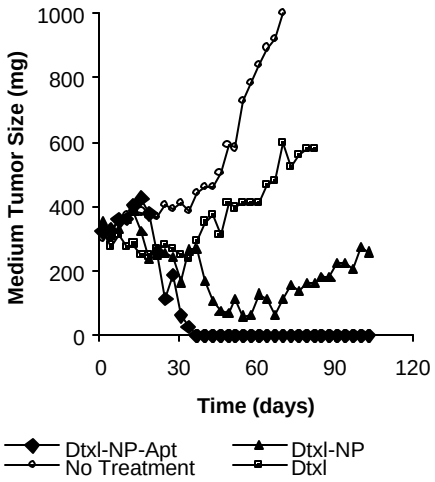

Figure 1. Antitumor effect of Dtxl-NP-Apt, Dtxl-NP, a Dtxl in nude mice bearing LNCaP tumor

CONCLUSION

Nanoparticle-aptamer bioconjugates show protracted and greater antitumor effect than nanoparticles without aptamer, suggesting that nucleic acid ligands may be utilized in targeted tumor therapy.

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SYNTHESIS AND CHARACTERIZATON OF NANOPARTICULATE CAP/CISPLATIN FOR LYMPHATIC TARGETED DRUG DELIVERY

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INTRODUCTION

Because many cancers (including breast cancer) mainly disseminate through the lymphatic route, it is very important to develop a drug delivery system which can not only help to treat the primary tumor, but also target the lymphatic tissue to kill metastatic cells. We hypothesize that nanoparticulate chemotherapy drug conjugates, utilizing a Calcium Phosphate (CaP) carrier, can target lymphatic tissue after subcutaneous injection, and thus treat or prevent cancer metastasis. Additional benefits anticipated from the CaP based nanoparticulate drug delivery system are reduced drug side effects and enhanced drug efficacy from the localized drug application.

EXPERIMENTAL METHODS

CaP nanoparticles were synthesized either by reverse microemulsion or by direct precipitation in the presence of polyelectrolytes at room temperature. Cisplatin was bound to the surface of the CaP through electrostatic interactions. These CaP nanoparticles and CaP/CDDP nano conjugates were characterized by transmission electron microscopy (TEM), particle size analysis, FTIR, x-ray diffraction (XRD), and atomic absorption spectroscopy. A near infrared imaging label was additionally conjugated to the nanoparticle surface. The CaP nanoparticles and conjugates of different sizes were then tested for optimal lymph node accumulation in a mouse model via near infrared imaging and lymphatic accumulation assay. The *in vitro* cytotoxicity of CaP/CDDP conjugates was assessed *in vitro* with the non-radioactive Cell Titer 96 Aqueous One solution cell proliferation assay using the 66cl4 mouse mammary carcinoma cell line.

RESULTS AND DISCUSSION

Calcium phosphate nanoparticles were successfully synthesized in three particle size distributions (10-50 nm, 100-1000 nm, and 1000-10,000nm). FTIR and XRD showed that the nanoparticles are mainly hydroxyapatite. TEM and particle size analysis revealed a uniformity of particle size. We find that the size of CaP nanoparticles and surface chemistry greatly affect the drug (CDDP) loading and the in-vitro drug release. The size of nanoparticles also has great influence on lymph node accumulation. In-vitro cytotoxicity test shows that the nanoparticulate conjugates have similar cytotoxicity (IC-50 value) as the control drug (free cisplatin), indicating no loss of drug activity due to conjugate to the CaP nanoparticulates. The anti-cancer effectiveness of the lymph node targeted CaP/CDDP nanoparticulates on primary tumor and lymph node metastasis will be tested further in the 66cl4 Mouse mammary tumor model

CONCLUSION

Calcium phosphate nanoparticles with controlled particle size were synthesized and conjugated with cisplatin and a near infrared imaging agent. These nanoparticulate conjugates have great potential for lymphatic targeted drug delivery to prevent cancer metastasis.

ACKNOWLEDGEMENT

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HPMA COPOLYMER-DOXORUBICIN CONJUGATES WITH PH-CONTROLLED ACTIVATION; EFFECT OF HYDROPHOBIC SIDE CHAINS

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INTRODUCTION

We showed recently that amphiphilic *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers containing anticancer drug doxorubicin (DOX) bound via pH-sensitive hydrazone bond exhibit high cytotoxicity for T cell lymphoma EL4 cells and a significant therapeutic effect in the treatment of EL4 lymphoma in mice (1,2,3). DOX is released from these conjugates in mild acid conditions simulating the environment of endosomes of tumour cells; on contrary hydrazone bond is relatively stable in neutral pH simulating conditions during transport in blood vessels. In this study we present synthesis of HPMA copolymer-DOX conjugates containing hydrophobic substituents in the polymer structure. All the conjugates were designed to form high-molecular-weight micellar structures enabling enhanced uptake in solid tumors due to EPR (enhanced permeability and retention) effect or improved interaction with tumor cell membrane followed by release of cytotoxic drug (DOX) in the target tumor cells.

RESULTS AND DISCUSSION

Here, we present synthesis and physico-chemical characterization of several polymer precursors and their conjugates with DOX, which differ in a structure and content of hydrophobic substituent. We synthesized copolymers – polymer precursors containing hydrazide groups and dodecyl or oleoyl aliphatic chains or highly hydrophobic cholesteryl group. Content of hydrophobic groups in the copolymers varied from 1 to 7 mol-% according to hydrophobicity of the substituents. The polymer precursors were then used for attachment of DOX to the HPMA copolymer chain via hydrazone bond.

Both the polymer precursors and the conjugates created micelles in 0.15 M saline solution. Average diameters of the micelles were about 12 – 35 nm and size distributions were relatively narrow. Also molecular weight of these micelles was about $0.5 – 3.5 \cdot 10^5$ g.mol^{-1}, while molecular weight (M_w) of single polymer chains was about $2 \cdot 10^4$ g.mol^{-1}. The higher was the content and hydrophobicity of the substituents, the higher was molecular weight and hydrodynamic radius of the micelles. Already Maeda showed that HPMA polymers effectively accumulate in solid tumors by the EPR effect (4) and that this accumulation strongly increases with increasing molecular weight of the polymer. We suppose that micellar systems with diameter of about 30 nm could be accumulated even more effectively in a solid tumor than earlier tested linear molecules. In addition, we expect that lipophilic substituents of the conjugates could interact with lipophilic part of membranes of the target tumor cells thus improving the transport and uptake of these conjugates inside the cells. Release of DOX from micellar drug carriers was verified in buffers at pH 5 and 7.4. All the micellar systems released DOX with the rate of 50%/24 h at pH 5 and 5% at pH 7.4 (phosphate buffer 37°C). It means, that micellar systems are relatively stable at pH of blood and efficiently release DOX in buffers modelling intracellular environment. Steric hindrances are probably responsible for decrease of the rate of DOX release from micellar systems by 20% if compared with simple polymer without main chain modification. Nevertheless, the rate of DOX release remains high enough to ensure sufficient intracellular DOX concentration evoking significant cytotoxic effect.

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PREPARATION AND CHARACTERIZATION OF PLGA NANOPARTICLES 9-NITROCAMPTOTHECIN, A NOVEL ANTICANCER DRUG, BY NANOPRECIPITATION METHOD

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INTRODUCTION:

The obstacle associated with cancer therapy is inefficient delivery of anticancer drugs to the tumors and sever toxicity due to their nonselective action. For overcome these problems one of the most important strategy is controlling both tissue and cell distribution profiles of anticancer drugs by their entrapment in submicronic colloidal systems (polymeric nanoparticles and liposomes) that can be enhanced permeability and retention (EPR) effect by increase selectivity towards cancer cells.
The main object of this study was to develop a polymeric drug delivery system for a new and potent antitumor drug, 9-Nitrocamptothecin (9-NC), intended to be intravenously administration and capable of improving the therapeutic index of drug.

EXPERIMENTAL METHODS:

To achieve this goal 9-NC loaded poly (DL-*lactide*-co-glycolide) (PLGA) nanoparticles were prepared by nanoprecipitation method. The full factorial experimental design was used to study the influence of four different independent variables, on response of nanoparticle drug loading. Analysis of variance (ANOVA) was used to evaluate optimized conditions for the preparation of nanoparticles. The physical characteristics of PLGA nanospheres were evaluated using particle size analyzer and scanning electron microscopy.

RESULTS:

The results of optimized formulations showed a narrow size distribution with a polydispersity index of 0.01%, an average diameter of 190 ± 20 nm and with drug loading of more than 30%. The in vitro drug release profile showed a sustained 9-NC release up to 160 h indicating the suitability of PLGA nanoparticles in controlling 9-NC release. The prepared nanoparticles as those described here may be clinically useful to stabilize and deliver camptothecins for the treatment of cancer.

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COMPUTER-AIDED MOLECULAR MODELING -TREND SETTING APPROACH IN THE DESIGN OF BIONANO DRUG DELIVERY SYSTEMS

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INTRODUCTION

Computer-aided molecular modeling (CAMM) is an important part of lead discovery and optimization, biological target identification, and protein/nucleic acid design. CAMM as a tool represents a trend setting approach in the design of polymeric nanoparticulate DDS (NPDDS). Polymeric NPDDS are an important armamentarium of bionanomedicine. A major limitation of NPDDS however, is drug loss during processing. Standard methods of optimization would entail a number of experimental runs and large drug quantities. This could therefore prove a serious limitation for high cost drugs and drugs discovered during HTS. Design of NPDDS of Doxo rubicin (water soluble) and Gliclazide (insoluble) using the CAMM approach has been demonstrated by the authors (1).The present study discusses CAMM as a predictive approach in the development of NPDDS of a hepatoprotective drug Silymarin (sparingly soluble).

OBJECTIVE

The objectives of the present study include:

(a) Predicting polymer-drug interaction to select the ideal polymer to maximize drug encapsulation.
(b) Predicting encapsulation efficiency (EE) for said drug-polymer combination.

EXPERIMENTAL

Silymarin a hepatoprotective, was selected as a model sparingly water soluble drug. Six polymers with varied functional groups namely, alginic acid, sodium alginate, chitosan, Gantrez AN 119, Eudragit L100 and Eudragit RSPO were selected for the CAMM study. The structures of the drug and polymers were built individually and their minimum energy conformations determined first using Molecular mechanics force field followed by AM1 Semi-Empirical Quantum mechanics and finally by *ab inito* method with minimal basis set. Various structural descriptors were evaluated. Cluster analysis, non linear regression and Artificial Neural Network (ANN) models were used to predict encapsulation efficiency. Drug loaded polymeric nanoparticles were prepared at a drug: polymer ratio of 1:2 and experimental encapsulation efficiency values determined.

RESULT AND DISCUSSION

CAMM study suggested that EE correlated directly with interaction energy and inversely with the hydration energy of the drug. Conclusive results were not obtained using cluster analysis. EE was predicted using a non-linear regression model. ANN approach to correlate predicted and experimental EE gave R^2, R^2_{adj} R^2_{pre} and F ratio of 0.9, 0.86, 0.56 and 126 respectively indicating a good model fit.

CONCLUSION

Computer-aided molecular modeling (CAMM) represents a trend setting approach in the design and development of NPDDS. This virtual approach could enable theoretical design and therefore additionally serve as an interface between New Drug Discovery and New Drug Delivery.

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EXTENDED RELEASE OF HYDROPHILIC MOLECULES FROM VESICLE-BIOPOLYMER GELS

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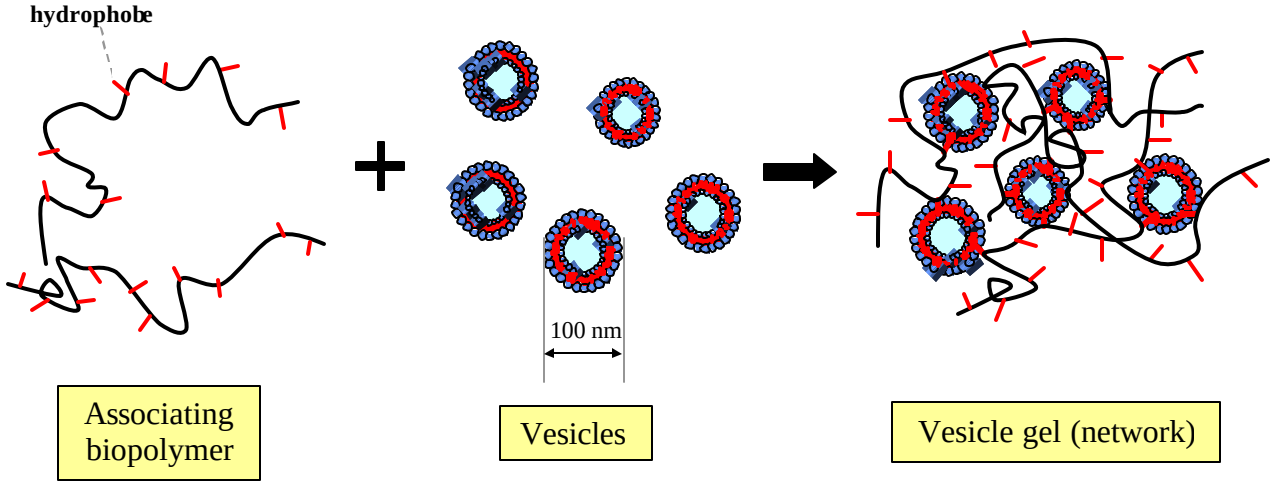
We study the release of the hydrophilic dye, calcein, from a gel formed by the noncovalent interactions of an associating polymer with surfactant and lipid vesicles. The associating polymer is obtained by attaching hydrophobic alkyl chains to the backbone of chitosan, a polysaccharide that has been used extensively in a wide range of pharmaceutical applications. Vesicle-polymer binding is expected to occur via the insertion of polymer hydrophobes into the vesicle bilayer, as depicted in Figure 1. Each vesicle thus acts as multifunctional crosslink in the network structure. This system may have a number of useful drug delivery applications because of its mechanical integrity and its extended release of solutes packaged within the vesicles. We have demonstrated that the presence of two transport resistances (i.e. (1) the hydrophobic bilayer and (2) the polymer matrix) in these vesicle gels significantly decreases the diffusion of calcein molecules encapsulated within the aqueous core of the vesicles. Addition of Triton-X100, a non-ionic detergent that dissolves surfactant and lipid vesicles, causes a sharp jump in UV-Vis absorption from an increased calcein concentration in external buffer media. This result suggests that the vesicles remain stable within the gel and that a large fraction of the packaged dye is retained after a period of several days. Lastly, this vesicle gel presents the variable of pH-sensitivity; for pH values less than about 6.0, enough of the free amine groups on the polymer backbone are protonated so that it becomes water-soluble. At this critical pH point, the mechanical robustness of the system is lost as the gel becomes a liquid, and diffusion of dye into external media distinctly increases. This property holds promise for targeted release applications in chemotherapy, as cancer cells have a low pH environment.



PROLONGED PROTECTION OF ENDOTHELIUM FROM OXIDATIVE STRESS BY TARGETING ANTIOXIDANT ENZYME-LOADED POLYMER NANOCARRIERS

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INTRODUCTION: Vascular oxidative stress is a ubiquitous, difficult to treat pathological condition. Targeting of antioxidant enzymes (e.g., catalase) to the vascular endothelium affords protection against reactive oxygen species (e.g., H_2O_2) in acute settings (1,2). However, lysosomal degradation of targeted enzymes significantly limits the duration of therapy (3,4). We have shown that AOE loading into degradable polymer nanocarriers (PNC) can prolong enzymatic activity by decelerating proteolytic degradation (5). In this study, we test the ability to lengthen the duration of endothelial protection by delivered enzyme through the use of targeted PNC.

EXPERIMENTAL METHODS: Poly(ethylene glycol)(5KDa)-block-Poly(lactic acid)(20 KDa) (PEG-PLA) PNC containing 15wt% Biotin terminated PEG-PLA were synthesized using previously established methods (5). EC targeting was achieved by decorating PNC with PECAM antibody-streptavidin conjugates that were prepared using NH_2-to-SH conjugation. Number of antibody molecules conjugated per PNC and PNC targeting to human umbilical vein endothelial cells (HUVEC) was determined by radiotracing. Antioxidant protection was tested by incubating HUVEC with anti-PECAM/PNC or IgG/PNC for 1 hour, washing unbound fraction and subsequent treatment with 5mM H_2O_2. H_2O_2 degradation was monitored using an enzymatic assay and cell viability was determined by ^{51}Cr release (6).

RESULTS AND DISCUSSION: Incubation of biotin-PNC with antibody-streptavidin conjugates resulted in antibody conjugation to PNC at a high surface density (6000±280 antibodies/μm^2) without aggregation. Antibody-coated, catalase-loaded PNC specifically bound to endothelial cells (e.g., 200±16.2 vs. 15±3.5 PNC/cell, anti-PECAM/PNC vs. IgG-PNC control). Targeting of catalase-loaded anti-PECAM/PNC, but not IgG/PNC, rapidly degraded H_2O_2 added to cells and completely protected HUVEC from injury. In order to determine delivered enzyme stability, cell-bound PNC were incubated in HUVEC for various times prior to H_2O_2 addition. Even after 3 hours incubation, only a 30% loss in cell-bound catalase activity and still complete protective effect were detected. In fact, ~20% of the catalase activity remained after 21 hours, resulting in 53.5±3.4% protection, corroborating the published result that a stable 25% level of enzyme remains loaded in degradable PNC. In contrast, effect of protease-sensitive anti-PECAM/catalase conjugates (7) displayed only 5-10% activity after 3 hours.

CONCLUSIONS: This work demonstrates for the first time the targeting of PNC loaded with an active antioxidant cargo, resulting in the complete protection of HUVEC from H_2O_2 toxicity. Finally, PNC prolongs the stability of delivered enzyme allowing for longer protection by a single dose.

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ENHANCED ANTI-TUMOR EFFICACY OF DOXORUBICIN-LOADED LONG-CIRCULATING LIPOSOMES MODIFIED WITH NUCLEOSOME-SPECIFIC MONOCLONAL ANTIBODY 2C5

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INTRODUCTION:

To enhance tumor targeting, doxorubicin-loaded long-circulating liposomes (Doxil®, Alza Pharmaceuticals) were modified with the monoclonal nucleosome-specific 2C5 antibody (mAb 2C5) that recognizes a broad variety of tumor cells via the tumor cell surface-bound nucleosomes. The 2C5-modified doxorubicin-loaded liposomes exhibited significantly increased target cancer cell killing *in vitro* and improved antineoplastic activity *in vivo* compared to the 2C5-free liposomes.

EXPERIMENTAL METHODS:

The clinically approved Doxil® was additionally modified with the mAb 2C5 antibody. Initially, mAb 2C5 antibody was modified with 40 molar excess of the p-nitrophenylcarbonyl activated poly (ethylene glycol)-phosphatidylethanolamine conjugate (pNP-PEG-PE). The 2C5-(PEG-PE) conjugate was incorporated into the liposome membrane by the post-insertion method. Tumor cell association at 4°C and 37°C was verified both microscopically and by FACS analysis of murine colon cancer (CT26), Lewis lung carcinoma (LLC), and breast cancer (4T1) cells, using fluorescently-labeled liposomes mimicking the lipid composition of Doxil®. Furthermore, ^{111}In-radiolabeled Doxil®-mimicking liposomal formulations were used to examine *in vivo* tumor accumulation of the different formulations. The cytotoxicity and therapeutic efficacy of 2C5-modified Doxil® were compared to various control liposomal preparations using different cancer types.

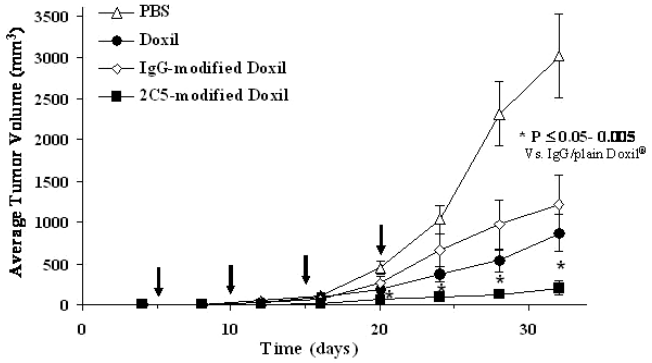

Figure 1. Therapeutic activity of 2C5-Doxil® against control preparations in **C26 (murine colon cancer)** implanted mice. Arrows indicate treatment schedule, **2.2 mg/kg/q 5 days**. (n = 8 - 10).

RESULTS AND DISCUSSION:

The 2C5-modified liposomes demonstrated superior *in vitro* cell binding and *in vivo* tumor accumulation, in LLC and 4T1 cancer models, compared to control IgG formulation. In addition, 2C5-targeted Doxil® demonstrated markedly higher *in vitro* anticancer activity and superior *in vivo* anti-tumor efficacy against various cancer models, mainly 4T1, LLC and CT26. The increased therapeutic efficacy of 2C5-targeted Doxil® formulation resulted in significantly smaller tumor volumes and weights compared to control Doxil® treatments (original or IgG-modified), using 4 doses of 2.2mg doxorubicin /kg/ q 5 days.

CONCLUSIONS:

Doxorubicin-loaded long-circulating liposomes were successfully modified with the mAb 2C5 antibody, specifically recognizing a wide variety of tumors. These tumor-targeted liposomal formulations displayed increased anticancer efficiency compared to the non-targeted analogs, thus exhibiting a potential for targeted anti-tumor therapy.

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DEVELOPMENT AND EVALUATION OF "SMART" POLYMER-ANTISENSE OLIGODEOXYNUCLEOTIDES COMPLEXES

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INTRODUCTION:

The objective of this research is to develop "smart", pH-sensitive, and membrane-destabilizing polymer-antisense oligodeoxynucleotides (ASODN) complexes that can escape the endosomal membrane and reach the cytoplasm of targeted cells. This report summarizes the formulation, characterization, *in vitro* evaluation, and *in vivo* toxicity and biodistribution of "smart" polymer-ASODN complexes.

EXPERIMENTAL METHODS:

The selected polymer composition is poly(PAA-*co*-BA-*co*-PDSA) terpolymer with a weight average molecular weight (M_W) of 14 KDa, which exhibits a pH-dependent, membrane-destabilizing activity in response to endosomal pH gradients (1). Cationic poly-L-lysine (PLL) chains with M_W 2.5, 10, or 48 KDa were grafted to this polymer backbone through serum-stable disulfide linkages to form "smart" polymer-PLL conjugates, which were used to complex a phosphorothioate ASODN (18 bases, 5699 Da) designed to block the pro-inflammatory IRAK-1 gene pathway in alveolar macrophages. The formulation of "smart" polymer-ASODN complexes was confirmed by the gel shift assay followed by measuring the size/zeta potential of the formed complexes using dynamic light scattering. The change in cellular uptake and sub-cellular distribution of free Alexa Fluor-labeled ASODN and "smart" polymer-ASODN complexes by THP-1 cells was examined using fluorescence microscopy. The compatibility of promising "smart" polymer-PLL conjugates was investigated *in vivo* as a function of the administered dose (20, 40, 60 mg/Kg body weight). *In vivo* biodistribution of free ^{3}H-labeled ASODN and its complexes with ^{14}C-labeled "smart" polymer-PLL conjugates was investigated as a function of time using the corresponding plasma profile, net accumulation in vital organs (heart, lungs, liver, kidneys, spleen), and excretion in urine and feces as key markers.

RESULTS AND DISCUSSION:

"Smart" polymer-PLL conjugates were synthesized and retained their pH-dependent, membrane-destabilizing activity after their complexation with therapeutic ASODN. However, the optimum N/P (+/-) ratio required to form stable complexes and the size/zeta potential of the formed complexes varied based on the length of the PLL graft. "Smart" polymer-PLL conjugates with PLL grafts of M_W 2.5, 10, and 48 KDa formed stable complexes with ASODN at N/P ratios of 8/1, 3/1, and 1/1, respectively. Similarly, the corresponding size/zeta potential of the formed complexes was 188 ± 16 nm/ -6.2 ± 2.9 mV, 602 ± 119 nm/11.1 ± 3.6 mV, and 892 ± 112 nm/16.7 ± 3.6 mV, respectively. The polymer-ASODN complexes incorporating PLL grafts of M_W 2.5 and 10 KDa were serum stable (> 75%) and caused no toxicity when incubated with THP-1 cells for 24 hours. Nano-sized polymer-ASODN complexes (< 200 nm) significantly increased cellular uptake and cytoplasmic distribution of the incorporated ASODN. Additionally, nano-sized polymer-ASODN complexes were highly biocompatible up to a conc. 60 mg/Kg body weight as shown in the histological examination of different organs. Free ^{3}H-labeled ASODN was rapidly cleared (< 0.5 hour) from the systemic circulation when administered intravenously, whereas "smart" polymer-ASODN complexes showed slower elimination from the systemic circulation and higher accumulation in target organ, the lungs. Cationic, polymer-ASODN complexes with 10 KDa PLL grafts, achieved significant accumulation in the lungs (26 ± 14 % of the administered dose) within 2 hours of their injection into the jugular vein, which declined to 8 ± 2% of the administered dose over a period of 24 hours. For anionic, polymer-ASODN complexes with 2.5 KDa PLL grafts, an average of 15 ± 5 % of the administered dose was retained in the lungs 24 hours after complex administration into the jugular vein. It is important to note that both ^{3}H-labeled ASODN and ^{14}C-labeled polymer-PLL conjugates showed similar distribution profiles when administered as pre-formulated complexes, which further confirms the *in vivo* stability of these complexes.

CONCLUSION:

Results show that "smart" polymers can form stable complexes with therapeutic ASODN, achieve high serum stability both *in vitro* and *in vivo*, and accumulate in target organs by varying their properties such as size/net charge.

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HPMA POLYMER CONJUGATES WITH DOXORUBICIN ATTACHED VIA HYDRAZONE BOND: IMPROVEMENT OF EFFICACY

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INTRODUCTION:

Recently we have shown that synthetic water-soluble copolymers of *N*-(2-hydroxypropyl)methacrylamide (HPMA) containing anti-cancer drug doxorubicin (Dox) bound via hydrolytically degradable linkage provide a high-potential drug-delivery system facilitating specific drug delivery to model tumors in mice[1]. In these conjugates, the drug is attached to the polymer carrier via a spacer containing a hydrolytically degradable hydrazone bond between the drug and polymer carrier (hydrazone conjugate). Such polymer-Dox conjugates are stable in aqueous solution at pH 7.4 (pH of blood) and Dox is released in mildly acidic environment (pH 5 - 5.5) (pH in endosomes of target cells). Original HPMA copolymers developed in our laboratory and used for the synthesis of hydrazone conjugates were water-soluble linear polymers with low molecular weight. The *in vivo* efficacy of such polymer-Dox conjugates in model tumors in mice was higher than that of free Dox, but not as high as we expected[2].

Here, we describe the synthesis and properties of new polymer-drug conjugates with enhanced content of the incorporated drug and containing structure units enabling improved interaction with tumor tissue and enhanced accumulation in solid tumors. Hydrophobic side chains were introduced into polymer structure to enable self-assembly of the copolymers into high-molecular-weight supramolecular structure allowing enhanced non-specific accumulation due to the EPR effect and oligopeptide sequences or cationic groups were incorporated to improve interaction of the conjugate with the target cell membranes.

RESULTS AND DISCUSSION:

Five conjugates differing in the amount of Dox were prepared with the aim to study the effect of drug loading on in vivo anti-tumor efficacy. The Dox content varied from 4 to 19 wt-% and all the conjugates were water-soluble at body temperature. Increasing the amount of Dox had no significant effect on its release profile from the conjugate incubated in buffers at pH 7.4 and 5. High-molecular-weight conjugates designed for enhanced accumulation in solid tumors were prepared by grafting the main polymer chain with semitelechelic polymers. Molecular weight of both the polymers (main chain, polymer grafts) was below the exclusion limit of kidneys. Semitelechelic grafts were attached to the main polymer chain via spacers susceptible to enzymatic or reductive degradation enabling intracellular degradation of the conjugate followed by elimination of the original polymers from the body. Incubation of the grafted high-molecular-weight conjugates with either cathepsin B or glutathione (3.10^{-3} mol/l) resulted in complete degradation after 24 h and formation of polymer products with molecular weights ca. 25 000.

Side chain structure units, i.e., oligopeptides (Gly-Gly-OH or Gly-Phe-Leu-Gly-OH), tertiary amino or quaternary ammonium groups were introduced into polymer chains by copolymerization with corresponding monomers. Introduction of a small amount of carboxylic groups into polymer structure resulted in an increase in the rate of Dox release at pH 5, while introduction of positive charges did not influence the rate of Dox release. Preliminary results of anti-tumor activity (mice, EL4 T-cell leukemia) showed increasing anti-tumor activity of graft conjugates and conjugates with higher drug content. Detailed biological evaluation is under way.

ACKNOWLEDGEMENT:

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MONODISPERSE NANOCARRIERS: NOVEL FABRICATION OF POLYMERIC NANOPARTICLES FOR BIONANOTECHNOLOGY

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The delivery of therapeutic, detection and imaging agents for the diagnosis and treatment of cancer patients has improved dramatically over the years with the development of nano-carriers such as liposomes, micelles, dendrimers, biomolecules, polymer particles, and colloidal precipitates. While many of these carriers have been used with great success *in vitro* and *in vivo*, each suffers from serious drawbacks with regard to stability, flexibility, or functionality. To date, there has been no general particle fabrication method available that afforded rigorous control over particle size, shape, composition, cargo and chemical structure. By utilizing the method we has designed referred to as **P**article **R**eplication **I**n **N**on-wetting **T**emplates, or **PRINT**, we can fabricate monodisperse particles with simultaneous control over structure (*i.e.* shape, size, composition) and function (*i.e.* cargo, surface structure). Unlike other particle fabrication techniques, **PRINT** is delicate and general enough to be compatible with a variety of important next-generation cancer therapeutic, detection and imaging agents, including various cargos (e.g. DNA, proteins, chemotherapy drugs, biosensor dyes, radio-markers, contrast agents), targeting ligands (e.g. antibodies, cell targeting peptides) and functional matrix materials (e.g. bioabsorbable polymers or stimuli responsive matrices). **PRINT** makes this possible by utilizing low-surface energy, chemically resistant fluoropolymers as molding materials and patterned substrates to produce functional, harvestable, monodisperse polymeric particles.

To demonstrate the potential and compatibility of **PRINT** for introducing "soft" molecular recognition moieties and/or valuable therapeutic agents into functional particles, we have encapsulated oligonucleotide and protein cargos within them to generate monodisperse "particle devices." We have incorporated fragile biological cargos and recognition agents, *i.e.* DNA, proteins (fluorescently-labeled avidin (MW 68 kDa)), and small anti-cancer agents (doxorubicin) into PEG nanoparticles using the simple, mild and general **PRINT** technique. We have arguably generated DNA delivery vectors that are themselves first generation "synthetic viruses" (monodisperse populations of shape-specific particles containing DNA). Furthermore, these biomolecule-containing particles could be used as nanoscale, shape-specific biosensors or next-generation therapeutic agents. We were able to confirm the encapsulation of the oligonucleotides by observing fluorescence from monodisperse particles using confocal microscopy (Figure 1).

PRINT has several distinct advantages over other vector fabrication techniques in that the particles are monodisperse and shape specific. In addition, no surfactants condensation agents, etc. are required.

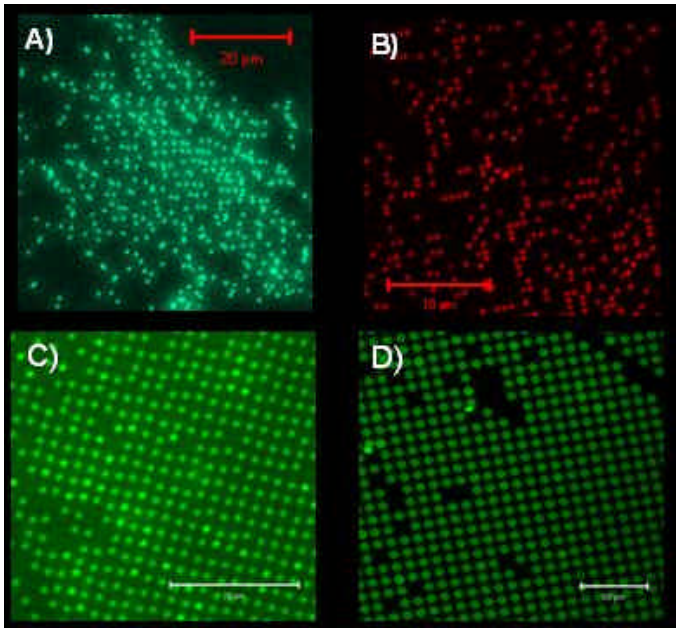

Figure 1. Confocal images demonstrating the control of the composition using **PRINT (PRINT** particles are all 500 nm conical PEG particles that are < 50 nm at the tip) A) encapsulated avidin (68,000 kDa); B) encapsulated 24-bp DNA; C) encapsulated doxorubicin; D) PEG particles tagged with a protein biosensor.

NANO-SCALE ENGINEERING AT THE CELL SURFACE: SYNTHESIS AND DELIVERY OF QUORUM SENSING AUTOINDUCER AT THE CELL SURFACE USING MAGNETIC NANOFACTORIES

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INTRODUCTION:

Functionalized magnetic nanoparticles are routinely used in a variety of applications in biomedicine. In many applications involving drug delivery, localized delivery is sought for improving therapeutic efficacy and decreasing harmful side effects. We hypothesize that the localized synthesis of a drug at the surface of a targeted cell and its subsequent uptake may reduce the amount of drug required and reduce side effects. To achieve this, we envisage the assembly of a magnetic nanofactory to synthesize the drug at the surface of a targeted cell. The nanofactory comprises of three components: a magnetic carrier for delivery and recovery, a precursor- or enzyme-containing modality that synthesizes the drug at the cell surface and a recognition/targeting domain (e.g., antibody) for delivering nanofactory to the appropriate cell surface. To demonstrate the concept, we synthesize the signaling molecule Autoinducer-2 (AI-2) at the surface of the bacterium *Escherichia coli* using a synthesized magnetic nanofactory. This nanofactory, demonstrated here for the first time, consists of an in vitro reconstructed biochemical pathway for the localized synthesis and delivery of the AI-2 signaling molecule. The AI-2 signal is transduced to modulate AI-2 specific gene expression in reporter *E. coli* cells.

EXPERIMENTAL METHODS:

Magnetic nanoparticles consisting of iron oxide and the biopolymer chitosan are prepared by co-precipitation (chitosan-mag).[1] The enzymes, Pfs and LuxS, used for the biosynthesis of AI-2 from S-Adenosylhomocysteine (SAH) are assembled onto chitosan-mag using the enzyme tyrosinase. This nanofactory is attached to the surface of *E. coli* ZK126 *?luxS* (which cannot intrinsically produce AI-2). Subsequently, the substrate SAH is added to the medium and AI-2 is synthesized at the surface of the cells via the nanofactory. The effect of the localized synthesis of AI-2 at the cell surface is monitored by measuring the expression of an AI-2 dependent reporter that produces β-Galactosidase.[2] The time rate of synthesis of AI-2 is carried out at different time points along the growth curve to check the effect of localized delivery on gene expression.

RESULTS:

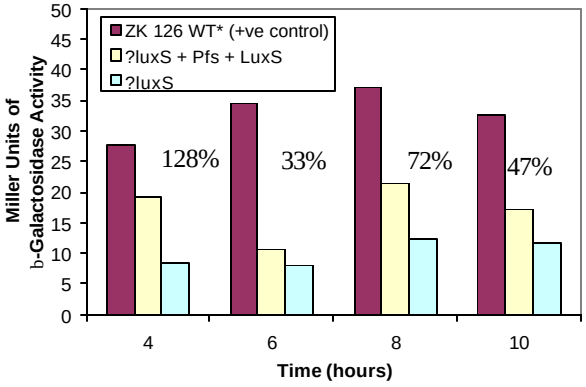

Figure 1. Synthesis of AI-2 at the cell surface increases β-Galactosidase expression (increase indicated in figure)

CONCLUSION:

Our nanofactories can be used to synthesize a molecule of interest (AI-2) at the cell surface. Localized synthesis of AI-2 results in increased expression of β-Galactosidase that demonstrates the concept of engineering at the cell surface

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MONOCLONAL ANTIBODY 2C5-MODIFIED LIPOSOMES SHOW ENHANCED ACCUMULATION IN SUBCUTANEOUS HUMAN BRAIN TUMOR XENOGRAFT IN NUDE MICE

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INTRODUCTION:

Brain tumors remain notoriously difficult to treat with the conventional tools of surgery, radiation and chemotherapy due to the existence of certain barriers in the brain (1). Liposome-based delivery systems have shown promising potential to deliver anti-cancer drugs within brain tumors (2). The modification of liposomes with monoclonal antibodies enhances the targeting of anti-cancer drugs to tumors. Monoclonal antibody 2C5 (mAb 2C5), an anti-nuclear autoantibody with nucleosome-restricted specificity, has been shown to be tumoricidal against various unrelated human tumors in nude mice (3). We hypothesized that subtherapeutic quantities of mAb 2C5 can be used for targeted delivery of liposomes to brain tumors *in vivo*.

The objectives of the study were to estimate the binding of mAb 2C5 to various human brain tumor cells: human astrocytoma U-118 MG, SW1088, CCF-STTG1 and U-87 MG, glioblastoma LN-18 and neuroblastoma SK-N-AS; to characterize the association of 2C5-immunoliposomes (2C5-ILS) with brain tumor cells *in vitro*; to examine the cytotoxicity of doxorubicin (Dox) loaded-2C5-ILS against brain tumor cells; and to establish subcutaneous brain tumor model in nude mice and assess the accumulation of 2C5-ILS in subcutaneous brain tumor *in vivo*.

EXPERIMENTAL METHODS:

The binding of mAb 2C5 towards brain tumor cells was tested by flow cytometry. Antibodies were coupled to the liposomes via *p*-nitrophenyl carbonyl-PEG_{3400}-PE linker lipid (4). The association of 2C5-ILS to brain tumor cells *in vitro* was confirmed by fluorescence microscopy. The cytotoxicity of Dox-ILS was investigated on the tumor cells using CellTiter 96® AQ_{ueous} One Solution Cell Proliferation assay. U-87 MG tumors were grown in nude mice subcutaneously. The tumor-bearing mice were injected with ^{111}In-labeled liposomes or ILS via the tail vein. Tumor and normal tissues were collected at different time points and ^{111}In radioactivity was quantified. Tumor-to-muscle and tumor-to-blood accumu lation ratios were calculated using % injected dose/g of tissue parameter.

RESULTS:

mAb 2C5 specifically recognized all tested brain tumor cells, the absolute intensity of this interaction being dependent on the cell type. 2C5-ILS displayed enhanced binding to the tumor cell surface compared to non-specific IgG-ILS. Dox-2C5-ILS were 9-14 fold more cytotoxic than non-specific Dox-IgG-ILS and control Dox-LS. *In vivo* studies on subcutaneous brain tumor revealed that the tumor-to-muscle accumulation ratio for 2C5-ILS and IgG-ILS was 14.65 ± 0.76 and 5.74 ± 0.79 respectively, post 24 h of liposomes injection (Fig 1). The tumor-to-blood accumulation ratio after 24 h of injection was 3.19 ± 0.36 and 2.05 ± 0.43 for 2C5-ILS and IgG-ILS respectively. 2C5-ILS thus showed significantly higher accumulation in subcutaneous U-87 MG brain tumors compared to normal tissue.

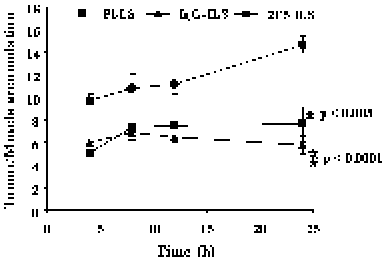

Fig 1. Tumor-to-muscle accumulation ratios for various liposomal preparations in nude mice with subcutaneously grown U-87 MG brain tumor.

CONCLUSIONS:

2C5-ILS show enhanced accumulation in subcutaneous brain tumor in nude mice model. 2C5-ILS can potentially be used for targeted delivery of anti-cancer drugs to brain tumors *in vivo*.

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ACKNOWLEDGEMENTS:

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GENETCALLY ENGINEERED SILK-ELASTINLIKE HYDROGELS FOR THE CULTURE OF HUMAN MESENCHYMAL STEM CELLS

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INTRODUCTION

Thermo-responsive biomaterials showing a sol-to-gel transition at body temperature are attractive candidates for applications related to regenerative medicine and tissue engineering [1]. Genetic engineering techniques allow the production of polymers with a high degree of control over sequence and length which can translate to control over gelation kinetics, biomechanics biocompatibility and biodegradation of the polymer. SELP-47K is a genetically engineered polymer composed of repeating blocks of silk (*GAGAGS*) and elastin (*GVGVP*) [2]. SELP-47K copolymers undergo an irreversible self-assembly at body temperature, allowing them to be potentially administered via minimally invasive procedures. In this study we evaluate the potential of SELP-47K as an injectable matrix for delivery of therapeutics such as human mesenchymal stem cells (*h*MSC).

EXPERIMENTAL METHODS

The cytotoxicity of SELP-47K polymer solution was evaluated in culture against *h*MSC using WST-1 assay. The cells were encapsulated in SELP-47K hydrogels prepared from 12 wt% polymer solution at a cell density of 2×10^6. Cell viability within the hydrogel was evaluated by WST-1 assay and by confocal microscopy using the Live/Dead assay kit. The production of matrix protein by the cell cultures in SELP-47K gels was qualitatively detected with RT-PCR analysis. The morphology and distribution of the encapsulated *h*MSC was examined histologically with H&E staining.

RESULTS AND DISCUSSION

In WST-1 cytotoxicity assay, the polymer solution showed no observable toxicity at a concentration up to 10 mg/ml. Detection of metabolic activity, analysis of synthesized mRNA and histological staining of *h*MSC cultures in these gels indicate cell proliferation without loss in metabolic activity at the three-week time point when cultured in basic growth medium.

CONCLUSIONS

The study suggests that SELP-47K is suitable for the *in vitro* proliferation of *h*MSC and can serve as an injectable carrier for cellular therapeutics.

ACKNOWLEDGENTS

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NANO-SIZE RECOMBINANT POLYMER/DNA COMPLEXES FOR TARGETED GENE DELIVERY

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INTRODUCTION

For therapeutic genes to reach the nucleus of target cells, the carrier should condense the DNA to protect it from nuclease degradation, be directed to the cells and internalized, allow escape from the endosomes and be directed towards the nucleus. A non-viral recombinant vector was designed comprised of lysine residues to condense pDNA into nano-size particles, histidine residues to disrupt endosome membrane via proton sponge hypothesis [1], and fibroblast growth factor (FGF2) to target cells over-expressing FGF2 receptors [2, 3]. The designed vector has the following structure: $(KHKHKHKHKK)_6$-FGF2.

METHODS

Cloning of (KH)6-FGF2 gene into the expression vector: Multimer gene segments encoding lysine–histidine repeats were cloned adapting seamless cloning technique for the synthesis of protein-based polymers [4]. The gene encoding FGF2 and (KH)6 were sequentially cloned into pET21b vector at its multiple cloning site and the cloned gene was sequenced by triple DNA sequencing and translated to its corresponding amino acid sequence.

(KH)6-FGF2 expression, identification, characterization and biological evaluation: pET21b-(KH)6-FGF2 expression vector was transformed into BL21(DE3) E.coli host and expressed. The expressed protein was characterized by SDS-PAGE, western blot analysis, MALDI-TOF and amino acid content analysis. The formation of the pDNA/vector complex was examined by agarose gel retardation assay. The mean hydrodynamic sizes of plasmid DNA/vector complexes were determined by Photon Correlation Spectroscopy. WST-1 assay was performed to evaluate the mitogenic activity and toxicity of $(KH)_6$-FGF2. The vector was complexed with pEGFP and its ability in presence and absence of serum to transfect NIH 3T3, T-47D, and COS-1 mammalian cells was examined.

RESULTS AND DISCUSSION

The fidelity of the construct to its original intended sequence was confirmed by DNA sequencing. The purified polymer showed >95% purity on SDS-PAGE. The molecular weight of $(KH)_6$-FGF2 was determined by MALDI-TOF to be 27,402 which was very close to the theoretical value of 27,313 daltons. The results of the amino acid content analysis agreed with the expected amino acid compositions. Gel retardation assays indicated that the vector does interact with DNA, retarding its migration and accessibility to ethidium bromide in a dose-dependent manner. The average particle size of the vector - DNA complexes, at a ratio of 1/100, was 231 ± 15 nm. The FGF2 motif present in $(KH)_6$-FGF2 was found to be active in terms of inducing cell proliferation in fibroblasts when they were exposed to concentrations of vector that mimicked physiological FGF2 levels (i.e., 0.02-50 ng/ml). The toxicity results revealed that $(KH)_6$-FGF2 did not have any deleterious effect up to 50 µg/ml on the cell proliferation rate. Transfection was observed in all cell lines, regardless of whether serum was present, though the percentage of transfected cells was significantly higher in the absence of serum.

CONCLUSION

It can be concluded that the $(KH)_6$-FGF2 vector is able to mediate gene transfer in various cell lines and constitutes an encouraging starting point for the biosynthesis of recombinant gene carriers with repetitive sequences for studying structure-transfection efficiency relationships. This work provides a platform for the subsequent biosynthesis of additional analogues where the ratio of Lys:His, the length of Lys-His repeats and the location of targeting moiety among other factors can be systematically changed by recombinant techniques.

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DIFFERENT ROLES OF PEPTIDIC SPACERS IN PROTEOLYTICALLY AND HYDROLYTICALLY CLEAVABLE HPMA-BASED POLYMERIC PRODRUGS

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INTRODUCTION:

Polymeric conjugates based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) have been tested as potential carriers for anticancer drugs, e.g. doxorubicin (Dox). Two types of these prodrugs differing in the way that doxorubicin is bound to the polymeric backbone were synthesized: a) prodrugs containing Dox bound through an amidic bond to an oligopeptidic side-chain (usually GFLG) [1,2], and b) hydrolytically cleavable prodrugs wherein Dox is bound to the polymeric carrier through a pH sensitive bond coupling the drug to an aminohexanoic acid as a spacer [3,4]. The mechanisms of action of both conjugates are substantially different and reflect the diverse ways and intensities of their intracellular internalization and destiny.

EXPERIMENTAL METHODS:

Incubation with polymeric prodrugs

$1x10^7$ cells (3T3 mouse fibroblasts) were either incubated (36 or 24 hrs) in a medium (RPMI1640, DMEM) containing tested conjugates (37 °C, 5 % CO_2 humidified atmosphere) or were just pulsed (90 minutes) with these conjugates at 4 °C. After the pulse, the cells were washed and chased in a fresh medium for 0-72 hours (37 °C, 5 % CO_2 humidified atmosphere).

Visualization of specimens and fluorescence quantification

The specimens were visualized using Olympus AX70-Provis fluorescence microscope and analyzed by AnalySIS software.

Quantitative analysis of fluorescence intensity in a particular cell compartment and apoptosis detection (Annexin V) was measured using a laser scanning microscope Olympus CompuCyte iCys.

RESULTS AND DISCUSSION:

The intracellular pathway of all tested conjugates could be tracked and quantified due to an intrinsic fluorescence of doxorubicin.

All conjugates containing doxorubicin bound via an amidic bond (either enzymatically cleavable (GFLG) or uncleavable (GG, LL) spacers) enter cells in a few seconds. These conjugates directly penetrate the plasma membrane and are detectable in all associated cellular membranes, i.e. membranes of the endocytic compartment, a nuclear membrane as well as membranes of Golgi and endoplasmic reticulum. We have never been able to detect released doxorubicin inside the nuclei of the treated cells. The cytotoxic effect depends on the rate of prodrug uptake which is strongly influenced by the sequence of peptidic spacer binding doxorubicin – GFLG sequence is supreme to all other spacers. The cytotoxicity of these conjugates seems to be primarily caused by the damage of cellular membranes. Necrosis is the main mechanism of the cell death [5].

Conjugates containing hydrolytically bound doxorubicin are internalized by endocytosis and fluid phase pinocytosis and are detectable inside the cells after a rather long period of time – 3 or more hours. Doxorubicin is cleaved from the polymeric carrier at low pH, i.e. in late endosomes and lysosomes. An apoptosis is the main mechanism of the cell death. The type of spacer influences the rate of the intracellular release of the drug rather than the rate of internalization of the prodrug.

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ACKNOWLEDGEMENTS:

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BREACHING THE BLOOD BRAIN BARRIER THROUGH AMINO ACID COUPLED LIPOSOMES

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INTRODUCTION: The effective delivery of the drug could be achieved by using various approaches.[1] Amino acid transport mechanism one of the strategies that can be utilized for the transport. Acidic amino acid transport system provides portals entry to the brain for glutamate and aspartate.[2] Therefore Glutamine coupled liposomes bearing dopamine-HCl were prepared to facilitate the transport of drug across BBB via receptor-mediated endocytosis.

EXPERIMENTAL METHODS: Equimolar quantities of stearylamine and L-glutamine were allowed to react with Dimethyl suberimidate (DMS) in the presence of 0.2 M triethanolamine buffer at pH 8.2 with continuous stirring for 30 min at room temperature. The resultant solution was then incubated for 8hr and reduced in vacuum desiccators to get precipitate. The precipitate was washed with water to remove any unconjugated glutamine and then was purified by dissolving and recrystallization in chloroform. Formation of conjugate was confirmed by IR-spectroscopic study of the L-glutamine stearylamine conjugate (GSC). Liposomes bearing dopamine HCl ware prepared by cast film method[3] using phosphatidylcholine (PC), cholesterol (CH) and GSC (for coupled liposomes).

Process parameters were optimized. Vesicle size determined using laser diffraction particle size analyzer; shape and surface were examined by transmission electron microscope. The drug entrapment efficiency was determined by disrupting the vesicles by Triton X-100. Formulations were subjected to in vitro drug release using dialysis membrane. The in vivo efficacy of the formulations was assessed by measuring the reduction in the degree of drug (Chlorpromazine) induced catatonia in albino rats. Fluorescent microscopy was performed to confirm the brain delivery of coupled liposomes.

RESULTS AND DISCUSSION: DMS was used as homobifunctional cross liking agent in the preparation of GSC as it contains amine reactive imidoester groups on both ends. IR spectrum of GSC exhibits the peaks at 3353 cm^{-1} and 1521 cm^{-1} that are due to N-H stretching and bending. Other peaks obtained further confirmed the conjugation. The average vesicle size of uncoupled and glutamine coupled liposomes were found to be in the range of 630± 25 nm to 588±21 nm and 678± 26 nm to 639± 24 nm, respectively. The small size of liposomes reduces the phagocytic uptake. The increase in the size of coupled liposomes could be due to the inclusion of GSC on the liposomal bilayer. The percentage encapsulation efficiency of uncoupled liposomal formulations was found to be 41.25±1.93% while it was decreased to 38.69±1.66% for coupled formulation due to change in bilayer statistics. The *in vitro* drug release after 24 hrs was 58.9±2.94% with uncoupled liposomes (LIP B) and 42.8±1.16% with coupled formulations (LIG B). The lower value for coupled formulation could be due to incorporation of GSC in the liposomal bilayer, which enhanced the structural integrity of the bilayer.

The *in vivo* efficacy of selected formulation was assessed by measuring the reduction in degree of drug-induced catatonia and by scoring the animals periodically. Difficulty in moving and changing the posture was evaluated by alternately placing the paw of the rat on a 3-cm and 9-cm high block. For a single rat, the maximum possible score was 3.5, which revealed total catatonia. The onset compared by observing the catatonia at 0, 15, 30 up to 180 min. Results revealed that dopamine-HCl solution did not show any reduction in degree of catatonia (score 3.5 after 180 min) while the uncoupled liposomes treated animals showed partial reduction on catatonia (score 3.5 for 4 animals and 1.5 for 2 animals). The animals that received coupled formulation showed almost complete reduction in catatonia (score 1.5 for 1 animal and 0.5 for 5 animals). This could be attributed due to the enhanced uptake of L-glutamine stearylamine coupled liposomes, which was further confirmed by fluorescence microscopy. Hence it could be concluded that the brain uptake of drug could be enhanced by using GSC coupled liposomes bearing drug.

Table: 1 Mean particle size and entrapment efficiency

Formulation	Variable lipid	Size(nm)	% Entrapment	Formulation	Variable lipid	Size (nm)	% Entrapment
LIP A	9:1	630±25	44.56±2.07	LIG A	8:2:1	639±23	28.26±1.03
LIP B*	8:2	610±24	41.25±1.93	LIG B*	8:2:1.5	650±25	38.69±1.66
LIP C	7:3	588±21	33.96±1.35	LIG C	8:2:2.0	678±26	37.75±1.54

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POLY (AMIDOAMINE) DENDRIMER PERMEABILITY AND CELLULAR LOCALIZATION IN CACO-2 CELL MONOLAYERS

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INTRODUCTION: Controlled drug delivery systems typically comprise a polymeric component that serves as a drug carrier to improve drug efficacy and reduce toxicity. However, the large size of most biopolymers limits their administration to the parenteral route. One class of polymers that demonstrates potential use as oral drug carriers is the family of PAMAM dendrimers. This research investigated the transport and cellular localization of a series of fluorescently labeled PAMAM dendrimers with positive, neutral and negatively charged surface groups in Caco-2 cell monolayers. Furthermore, the influence of PAMAM dendrimers on cellular tight junctions was examined using confocal microscopy.

EXPERIMENTAL METHODS: Cationic PAMAM-NH_2 (G2), neutral PAMAM-OH (G2), and anionic PAMAM-COOH (G1.5-G3.5) dendrimers were labeled with fluorescein isothiocyanate (FITC) using a 1:1 molar ratio. The permeability of fluorescently labeled PAMAM dendrimers of donor concentration 1.0 mM was measured across Caco-2 cell monolayers over 120 minutes in the apical-to-basolateral (AB) direction. ^{14}C-mannitol (3.2 μM) permeability was measured in the presence of unlabeled and FITC-labeled PAMAM dendrimers. Caco-2 cells were incubated with PAMAM dendrimers followed by anti-mouse occludin, then visualized using confocal laser scanning microscopy (CLSM) to examine the influence of PAMAM dendrimers on cellular tight junctions. The subcellular localization of G2NH_2- and G2.5COOH-FITC was observed using CLSM techniques.

RESULTS AND DISCUSSION: The overall rank order of PAMAM permeability was G3.5COOH > G2NH_2 > G2.5COOH > G1.5COOH > G2OH. ^{14}C-mannitol permeability significantly increased ($P < 0.05$) in the presence of cationic and anionic PAMAM dendrimers. The overall rank order of ^{14}C-mannitol permeability in the presence of PAMAM dendrimers was G2NH_2 > G1.5COOH > G2OH. Cells pretreated with G2NH_2 and G1.5COOH displayed disruption in the occludin staining pattern, as well as accumulation of occludin within the borders of cells. The observed increase in occludin expression indicates the opening of tight junctions when treated with G2NH_2 and G1.5COOH dendrimers. Diffuse, perinuclear staining was observed for transferrin-FITC, G2NH_2-FITC and G2.5COOH-FITC. Since transferrin is a known endocytosis marker, the confocal studies suggest G2NH_2 and G2.5COOH are also endocytosed.

CONCLUSIONS: PAMAM dendrimer permeability is dependent on structural properties including size and surface charge. The observed increase in ^{14}C-mannitol permeability due to FITC labeling suggests the attachment of hydrophobic drug compounds will affect drug-dendrimer permeability. Confocal studies suggest cationic and anionic PAMAM dendrimers are transported via an endocytosis route and enhance paracellular permeability. The reported data demonstrates the potential of PAMAM dendrimers as drug carriers and absorption enhancers to improve the transepithelial transport of poorly bioavailable molecules.

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ORAL DELIVERY OF INSULIN PLASMID USING CHITOSAN NANOPARTICLES

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INTRODUCTION

Oral gene therapy aims to treat immune-, gastrointestinal- or systemic diseases by ingestion of genetic materials. Compared to the other routes of administration, the oral route is the most convenient, but also the most challenging for gene delivery. Previous studies have shown that oral tolerance induced by administration of insulin, an autoantigen of type 1 diabetes, can induce a form of oral tolerance that prevents occurrence of disease in non-obese diabetic (NOD) mice [1]. In comparison to protein administration, delivery of pDNA-polymeric complexes has the potential for enhanced stability in the gastrointestinal tract and prolonged effect. This may lead to an enhanced mucosal immune response and reduced frequency of administration. Here we used a murine model to study the effect of administering chitosan nanocomplexes, containing pDNA encoding human insulin, on prevention of diabetes and longevity; and the effect of pDNA dose on human insulin blood levels.

EXPERIMENTAL METHODS

Female NOD mice, which spontaneously develop diabetes at age 12-20 weeks, were used. Urine glucose levels were measured twice a week. Treatment was initiated promptly on the detection of glycosuria (appearance of glucose in the urine). The study comprised four groups: (a) chitosan nanoparticles prepared by ultrasonication; (b) chitosan nanoparticles prepared by vortexing; (c) insulin protein; (d) untreated animals. The chitosan DNA-nanoparticles or the insulin protein embedded in gelatin cubes, were offered daily for five days (Fig. 1). In a second experiment (Fig. 2) chitosan nanoparticles containing pDNA doses of 0.1, 0.5. 1 and 2 mg, were administered once to 3-week old mice. Blood was drawn weekly thereafter and the human insulin concentrations in plasma were measured using ELISA.

RESULTS AND DISCUSSION

Figure 1: Effect of formulation of insulin DNA-chitosan nanoparticles on glycosuria of NOD mice

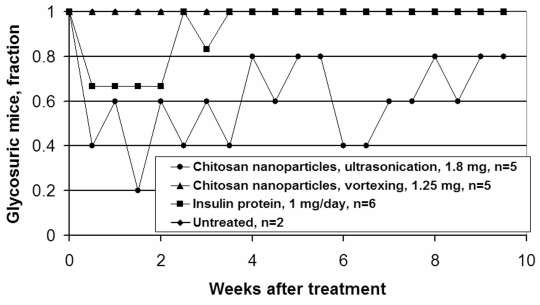
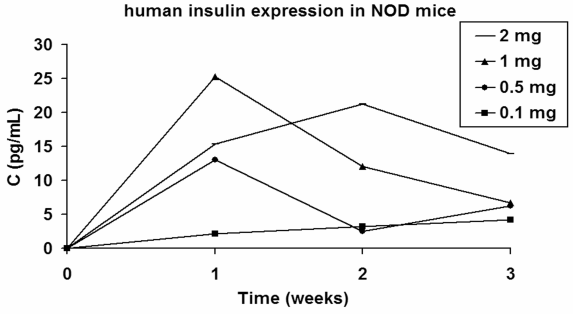


Figure 2: Effect of dose of human insulin plasmid administered as chitosan DNA-nanoparticles on human insulin expression in NOD mice



In one experiment, chitosan nanoparticles prepared by ultrasonication were more efficient than other formulations in temporarily reversing glycosuria. This pharmacological effect was seen in 4/5 mice (Fig. 1). This formulation also extended the longevity of the animals by more than one and two months in comparison to the vortexed chitosan nanoparticles and to the untreated group, respectively. In the second experiment, human insulin levels were detectable but low, in the range of 5-30 pg/mL (Fig. 2). These levels are about 10% of the average basal insulin levels in mice.

CONCLUSIONS

Oral gene delivery of chitosan nanocomplexes with insulin-encoding pDNA resulted in temporary reversal of glycosuria and prolonged longevity of NOD mice. The levels in the blood of the transgenically encoded protein were low but detectable.

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PREPARATION AND CHARACTERIZASTION OF ANTIBODY LABELED MAGNETIC IRON OXIDE NANOPARTICLES FOR BIOSEPRATIONS

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Modification of magnetic nanoparticles with active biomolecules is a promising approach to achieving sensitive separations. Here we demonstrate the efficient conjugation of the anti-mouse IgG antibody protein via glutaraldehyde activation of the amine-modified surfaces of magnetic iron oxide nanoparticles. Magnetically assisted bioseparations were carried out and fluorescence assays were used to establish loading capacity and activity of the immobilized antibody. A 1:1 binding model of the immobilized secondary antibody with the target primary antibody was established.

SEQUENTIAL CHANGES IN SALT CONDITIONS DURING THE HK: PLASMID FORMATION MARKEDLY AUGMENT TRANSFECTION EFFICIENCY.

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INTRODUCTION

In previous studies, we have determined that histidine-lysine (HK) peptides are effective carriers of plasmids *in vitro* (1,2). In HK peptides, the role of lysines is to bind to the negatively charged DNA phosphates while the role of histidines is to buffer the acidic endosomes and aid in DNA release from the endosomes. Dependent on the number of branches of the HK peptides, these peptides alone or in combination with cationic liposomes increase transfection efficiency. We investigated whether various salt conditions during formation of the HK: DNA complex affect transfection.

EXPERIMENTAL METHODS

Branched HK peptides (10 μg) in complex with a reporter-containing plasmid (2 μg) were formed in various media: 1) in Opti-MEM for 45 min (50 ul); 2) in water (50 μl) for 45 min; 3) in water (50 μl) for 30 min, followed by Opti-MEM (50 μl) for 15 min; and 4) in water (50 μl) for 30 min, followed by adding NaCl solutions (50 μl) varying from 100 for 600 mM. The complexes were added to medium containing the cells in 24 multi-well plates and reporter activity was measured as described previously (2). The size of the polyplexes was measured by N4 plus Submicron Particle Sizer and the surface charge was determined by the Delsa 440 SX zeta potential analyzer.

RESULTS AND DISCUSSION

HK peptides in complex with plasmid DNA were previously formed in Opti-MEM medium prior to adding the polyplexes to cells. When HK peptides in complex with a luciferase-containing plasmid complex were first formed in water for 30 min before 300 mM NaCl was added (final NaCl concentration 150 mM) for an additional 15 min, luciferase activity increased by 5-fold in 3 of 4 cells lines compared to when the polyplexes were formed in Opti-MEM. If the HK: luciferase-containing plasmid complexes were formed only in water, then luciferase activity was decreased compared to when the complexes were formed in Opti-MEM. By varying the salt conditions during formation of the HK:DNA complex, luciferase activity in several cell lines exceeded in 10^{10} RLU/mg-protein. Furthermore, sequential changes in salt conditions during the formation of the HK: β-galactosidase plasmid complex augmented β-galactosidase expression. In contrast, sequential changes from low to high salt did not affect the luciferase expression when DOTAP liposomes were carriers of luciferase containing plasmids. The HK polyplexes, formed in sequential salt conditions were intermediate in size (789nm ±56) compared to polyplexes formed in water (80nm±25) and Opti-MEM (1650nm ±310). The surface charge varied only slightly dependent on the salt conditions in which the HK: DNA complexes were formed.

CONCLUSIONS

HK: plasmid DNA complexes prepared first in water and then the NaCl concentration was adjusted to at least 150 mM had marked increases in luciferase and/or β-galactosidase activity in the majority of tested cells. Variation in the salt during formation of the HK:DNA complexes may increase transfection due to the size and the DNA release from the polyplexes.

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PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A LIPID-BASED FORMULATION OF A HYDROPHOBIC ANTI-CANCER AGENT

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INTRODUCTION

Efforts in medicinal and combinatorial chemistry continue to give rise to a wide range of anti-cancer agents with great therapeutic potential. However, many of these agents have solubility, stability or toxicity issues that retard or prevent their development into viable treatment strategies. In some cases, formulations or delivery vehicles can provide a means to fully exploit the therapeutic potential of these drugs. In this study a cholesterol-free liposome formulation was optimized and evaluated for delivery of a novel anti-cancer agent ML220 (i.e. 2-(5-bromo-1H-indol-3-yl)-1H-phenanthro [9,10-d] imidazole). ML220 is highly lipophilic with a water solubility of 0.14 μg/mL and calculated log P of 5.69. It has been demonstrated to have potent anti-proliferative activity against a variety of human cancer cell types [1, 2]. The main anti-cancer mechanism for this agent has been identified to include the induction of a partial arrest in the G_0/G_1 phase of the cell cycle and inhibition of kinase activity [1, 2]. However the pre-clinical evaluation of ML220 has been limited due to its low water solubility and high protein binding affinity.

EXPERIMENTAL METHODS

The liposome formulations formed from mixtures of egg phosphatidylcholine (ePC) and poly (ethylene glycol) conjugated distearoylphosphatidyl ethanolamine (DSPE-PEG 2000) were initially optimized in terms of the lipid concentration and the drug to lipid ratio. The optimized formulation (ePC : DSPE-PEG 2000 = 4:1 mol/mol, ML220 : total lipids= 1:10 w/w) was then prepared by a high-pressure extrusion method followed by lyophilization. The physico-chemical properties of this liposome formulation were evaluated in terms of drug loading properties, size, size distribution and stability. The in vitro anti-cancer activity of the formulated agent was examined in various cancer cell lines. The subacute toxicity of blank liposome and liposome-formulated drug was also investigated in C3H mice with doses from 0 to 100mg/ kg (ML220) for three consecutive weeks. The pharmacokinetics of both carrier (i.e. liposome) and liposome-formulated drug was studied in Balb/C mice. Finally the *in vivo* anti-cancer activity was evaluated in a human colon HT29 carcinoma xenograft model in CD-1 nude mice.

RESULTS AND DISSUSION

The optimized ePC/DSPE-PEG 2000 liposome formulation consisted of a unimodal population of vesicles with a mean diameter of 89 nm and provided more than a 50,000-fold increase in the water solubility of ML220. The drug to lipid ratio in the formulation was 1 : 3.5 (mol :mol), the drug loading efficiency was 83 % and the final concentration of solubilized drug was 8.3 mg/mL. The formulation was demonstrated to be stable *in vitro* at 37°C for over two weeks with release of less than 50 % of the total drug loaded. Evaluation of the subacute toxicity of ML220 administered to C3H mice in the liposome formulation revealed no overt signs of toxicity over a three-week treatment period. Intravenous administration of a 75 mg/ kg dose of the lipid formulated ML220 in Balb/C mice resulted in a biexponential drug plasma concentration pattern and the plasma concentration-time curve was best fit using a two-compartment model. The terminal half-life for ML220 was found to be 4.1 hours while the area under the curve was 1447 mg·h/ L. *In vivo* evaluation of anti-cancer activity in a human colon HT29 carcinoma model in nude mice revealed that treatment with the liposome formulated drug provided a significant delay in tumor growth, when compared to the saline control group.

CONCLUSION

These studies demonstrate that the fluid-like, cholesterol-free ePC-based liposomes have an especially high solubilization capacity for this drug. These preliminary findings are promising and support further evaluation of this formulation for ML220 and its hydrophobic analogues. These studies also highlight the potential of cholesterol-free liposomes as a formulation strategy for highly lipophilic drugs.

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INFLUENCE OF SERUM PROTEIN ON POLYCARBONATE-BASED COPOLYMER MICELLES AS A SYSTEMIC DELIVERY SYSTEM FOR HYDROPHOBIC ANTI-CANCER AGENT

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INTRODUCTION

Interactions with serum protein have been demonstrated to be one of the key factors that influence the *in vivo* fate of both colloidal carriers (e.g. liposomes, micelles and nanoparticles) and encapsulated active agents . It is postulated that there are two primary mechanisms by which protein may influence the *in vivo* fate of drug encapsulated in these colloidal carriers. Firstly, proteins may adsorb to the surface of these particles within the first few minutes of exposure and therefore influence the biological performance of the carrier. Secondly, if the drug has a high affinity for protein, the protein may accelerate the release rate of the drug from the particles. The protein bound drug will likely be cleared more rapidly from the circulation reducing the degree of accumulation at the tumor site, in comparison to vehicle-loaded drug. Therefore in this study, it is of interest to understand the extent to which serum protein influence the biological performance of block copolymer micelles as systemic drug delivery carriers.

EXPERIMENTAL METHODS

Novel amphiphilic polycarbonate-based diblock copolymers were synthesized with monomethoxy-terminated poly(ethylene glycol) (MePEG) as the hydrophilic block and poly(5-benzyloxy-trimethylene carbonate) (PBTMC) as the hydrophobic block. The copolymers were used to form micelles and investigated as a delivery system for the hydrophobic anti-cancer agent, ellipticine. Specifically, ellipticine was encapsulated into the micelles by a high-pressure extrusion method and the physico-chemical properties of the formulation were characterized. In order to study the interaction with serum protein, the extent of protein adsorption to the MePEG-*b*-PBTMC micelles was investigated by transmission electron microscopy, dynamic light scattering and gel filtration chromatography. The partitioning properties of ellipticine between an aqueous medium containing protein and the MePEG-*b*-PBTMC micelles were examined over a range of protein concentrations. The release kinetic profile and anti-cancer activity of drug-loaded micelles were studied and compared in the absence and presence of physiologically relevant concentrations of protein.

RESULTS AND DISCUSSION

In summary, block copolymers with controllable molecular weight and narrow molecular weight distributions were prepared with the use of triethylaluminum as precursor initiator and MePEG as the macroinitiator. The copolymers were demonstrated to be biodegradable and non-cytotoxic (up to 5g/L). The ellipticine-loaded MePEG-*b*-PBTMC micelles have a spherical morphology with an average diameter of 80 nm. Comparison of the micelle morphology and size prior to and following incubation with protein demonstrated that the amount of protein both loosely and tightly associated with the micelles was minimal and insignificant. The apparent partition coefficient of ellipticine between an aqueous medium containing protein and the MePEG-*b*-PBTMC micelles has been demonstrated to have a linear relationship with the mole ratio of protein to micelle. In this way, it was predicted that in the presence of physiologically relevant concentration of serum protein, 61% of the drug remained within this micelle system while 39% was in the protein-containing aqueous phase. In addition, the in vitro drug release profile of ellipticine from the micelles was fit using a modified Higuchi model and found to be accelerated in the presence of protein.

CONCLUSION

These studies demonstrate that although there are no significant interactions between this micelle system and protein, the properties of the micelle as a delivery vehicle may be strongly influenced by protein-drug interactions. The findings in these studies also highlight the importance of examining the characteristics of the formulation in the presence of physiologically relevant concentrations of plasma protein.

POLYMER-PEPTIDE CONJUGATES FOR TUMOR RADIOTHERAPY

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INTRODUCTION: Tumor cells rely on an intact vasculature for their survival[1], making the tumor vasculature an attractive target for therapy[2,3]. We evaluated the effectiveness of β-emitter radiotherapy delivered by polymer-peptide conjugates that bind to tumor angiogenic vasculature. This molecularly targeted radiation is intended to damage both the endothelial bed and surrounding neoplastic cells.

EXPERIMENTAL METHODS: *N*-(2-hydroxypropyl) methacrylamide (HPMA), a biocompatible and water-soluble copolymer[4] was derivatized to incorporate side-chains for ^{99m}Tc and ^{90}Y chelation and was further conjugated to a $\alpha_V\beta_3$ integrin targeting peptide (RGD4C). The HPMA copolymer-RGD4C conjugate was characterized by its side-chain contents, *in vitro* endothelial cell adhesion assay and its biodistribution and anti-tumor effectiveness in a SCID mouse xenograft model of human prostate carcinoma (DU145).

RESULTS and DISCUSSION: The conjugate contained about sixteen RGD4C moieties per polymer backbone. HPMA copolymer-RGD4C conjugates and free RGD4C caused similar degrees of inhibition of endothelial cell adhesion to fibrinogen at equivalent molar concentrations of RGD4C indicating no loss of peptide bioactivity upon conjugation to the copolymer. Tumor accumulation of the conjugate in SCID mice increased significantly ($p < 0.01$) over time from 1.05 ± 0.03% injected dose (%ID)/g tissue at 1 h to 4.32 ± 0.32% at 72 h. The activity in major normal tissues (blood, liver, spleen and kidney) decreased significantly ($p < 0.05$) during that period. At 21 days, the control tumors increased in volume from baseline by 442%. In contrast, a 7% and 63% decrease of tumor volume was observed for the 100 μCi and 250 μCi ^{90}Y treatment groups, respectively. Histopathological examination revealed increased apoptosis in the treated tumors with no signs of radiation induced toxicity to the kidneys, liver and spleen of the treated mice.

CONCLUSION: This copolymer-peptide conjugate provides a molecular delivery system that effectively targets the angiogenic sites and brings sufficient therapeutic radioisotopes within range to arrest tumor growth.

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ICAM-1-TARGETED NANOCARRIERS DIRECTED TO ENDOTHEIAL CELLS

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INTRODUCTION. Targeting drugs to endothelial cells (ECs) lining the lumen of blood vessels may optimize treatment of vascular disorders (reviewed by 1,2). The adhesion molecule ICAM-1, constitutively expressed by ECs and up-regulated by pathological factors including pro-inflammatory cytokines (e.g., TNFα and LPS endotoxin), represents an attractive marker for endothelial targeting (3). ICAM-1 targeting by multivalent anti-ICAM nanoconjugates provides delivery of therapeutic compounds (fibrinolytics, antioxidants, and lysosomal enzymes) to ECs (4-6). In this study we tested key parameters of affinity interactions of anti-ICAM nanocarriers (anti-ICAM/NCs) with EC *in vitro* and *in vivo*.

EXPERIMENTAL METHODS. As prototype nanocarriers we utilized FITC-labeled 100 nm polystyrene beads coated with anti-ICAM. Binding of beads to TNFα activated ECs (cultured under static conditions or adapted to flow at 9 dyn/cm^2) was determined by fluorescence microscopy (5). To evaluate targeting *in vivo*, uptake of ^{125}I-labeled anti-ICAM/NCs was analyzed and expressed as percent of injected dose per gram (%ID/g) in organs after IV injection in control or LPS-treated C57Bl/6 mice (4). To estimate the potential practical utility of this drug delivery system, we also tested endothelial targeting of anti-ICAM-coated biodegradable PLGA nanoparticles (7) produced by solvent extraction emulsification procedure.

RESULTS AND DISCUSSION. Anti-ICAM/NCs, but not control IgG/NCs, specifically bound to activated ECs (271±32 vs. 2±1 beads bound/cell). Binding of anti-ICAM/NCs to ECs was rapid at both static and flow conditions ($t_{1/2}$=5 min) and dependent on anti-ICAM density on the bead surface and dose of anti-ICAM/NCs. Anti-ICAM/NCs and IgG/NCs were cleared from circulation 30 min after injection in mice (4±0.4 and 6±0.4 % ID), predominantly by liver (47±3 and 51±4 % ID/g) and spleen (63±5 and 99±8 %ID/g). However, anti-ICAM/NCs specifically accumulated in pulmonary endothelium (100±10% vs. 9±2% ID/g). Lung targeting almost doubled in LPS-injected mice. Moreover, pulmonary targeting of anti-ICAM/NCs was proportionally modulated by antibody surface density, and further elevation of the injected dose augmented pulmonary targeting (46±14%, 117±20%, and 185±24% ID/g lung for $4.5x10^{10}$, $4.5x10^{11}$ and $1.3x10^{12}$ beads administered/mouse). Biodegradable PLGA polymer nanocarriers similarly showed high targeting efficacy to endothelium, both in culture (74±15 particles/cell) and in mice (180±20% ID/g in lung).

CONCLUSIONS. These data: (i) Indicate that multimeric anti-ICAM nanocarriers provide rapid, specific and efficient targeting to ECs, particularly to pathologically challenged ECs; and (ii) Identify key targeting parameters that will guide further design and optimization of drug delivery systems targeting ICAM-1.

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ENHANCED NUCLEAR IMPORT OF PLASMID DNA AND INCREASED EXOGENOUS GENE EXPRESSION USING STREPTAVIDIN-FUSED IMPORTIN-b

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INTRODUCTION

Over the past decade great deal of effort has been directed towards the development of effective non-viral vectors. Because the transfection efficiency of non-viral vectors is much lower than that of viral vectors, often due to the limited nuclear import of the transgene, many studies have focused on overcoming this problem.

Previous reports have suggested that the charge interaction between anionic DNA and cationic NLS renders the NLS inaccessible by burying it in the DNA. Furthermore, importin-β has been shown to play an essential role in nuclear import by interacting with both the NLS and the nuclear pore complex, and by delivering the protein into the nucleus. We hypothesized that the direct conjugation of importin-β to the plasmid DNA complex may significantly increase the nuclear import efficiency of plasmid DNA complex. To test this hypothesis, herein, we prepared plasmid DNA/importin-β conjugates consisting of biotinylated polyethylenimine (b-PEI) and recombinant streptavidin-fused importin-β (GβS). Moreover, the enhancement of nuclear entry of plasmid DNA and exogenous gene expression comes the scope of this paper.

RESULTS AND DISCUSSION

We examined whether the recombinant protein that contains green fluorescent protein, mouse importin-β (1-643 aa), and streptavidin (1-170 aa) could enter into the nucleus by microinjecting the protein into the cytoplasm of NIH3T3 cells. The recombinant protein (GβS) localized in the nucleus and nuclear membrane one hour following microinjection. On the other hand, in the presence of WGA lectin that is inhibitor of the transport through the nuclear pore complex, the import of fused protein is inhibited. This result indicated that nuclear import of streptavidin-fused importin-β is achieved through the nuclear pore. With Rhodamine-labeled Plasmid DNA/importin-β polyplex, nuclear colocalization of plasmid DNA and streptavidin-fused importin-β suggested that the importin-β attached to the plasmid DNA and delivered it into the nucleus. In contrast there was no plasmid DNA stimulation of nuclear localization without fused protein.

Transfection efficiencies of NIH3T3 cells by combination of nuclear importable polyplex with Sendai viral envelope vector (HVJ-E) were estimated by luciferase assay. Without HVJ-E, b-PEI hardly shows significant transfection efficiencies for NIH3T3 cells. Kaneda et al. have reported that HVJ-E vector is useful for both *in vitro* and *in vivo* gene expression because fusogenic HVJ-E delivers genes efficiently into cytoplasm by envelope-cell fusion. In our assay, when pGL3 was incorporated into HVJ-E luciferase expression was 13 times higher without HVJ-E. Incorporation of pGL3/PEI polyplex (N/P=10) enhanced transfection as similar to results with protamine sulfate. The increase in gene expression with PEI showed about 4-fold. Furthermore, to estimate the effect of nuclear import ability by GβS, 6.25% of PEI was substituted with b-PEI. When GβS was added into pGL3/b-PEI polyplex, expression was more 8.7 times higher in comparison with the level of expression in the absence of GβS.

The enhanced expression efficiency achieved by the importin-β/pGL3/b-PEI conjugate incorporated into HVJ-E is in accordance with the observed improvement in the nuclear import of GβS.

CONCLUSION

We show that novel HVJ-E/importin β hybrid vector could overcome plasma and nuclear membrane barriers to achieve effective delivery of a plasmid DNA/PEI complex into the nucleus.

ACKNOWLEDGEMENTS

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LIPOSOME TARGETING OF COMBRETASTATIN TO IRRADIATED TUMORS RESULTS IN TUMOR GROWTH CONTROL

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INTRODUCTION

Ionizing radiation has been shown to upregulate various adhesion molecules in normal vessels (1). Upregulation of adhesion molecules on the luminal surface of vessels in tumors irradiated for therapeutic purposes presents an opportunity for selective targeting of antivascular drugs to tumors.

EXPERIMENTAL METHODS

The antivascular drug combretastatin was incorporated into liposomes (approximately 120 nm) with surfaces modified by the addition of cyclo(Arg-Gly-Asp-D-Phe-Cys) (RGD), to create a targetable liposome (TL). This addition of RGD allows the liposome to be preferentially targeted to avß3, an integrin upregulated in the vasculature of irradiated tumors. C57BL mice bearing a transplanted B16-F10 melanoma were randomly assigned to one of the following treatment groups: untreated, a single dose of 5 Gy radiation (TR), TL (14.5 mg/kg of combretastatin), 5 Gy radiation plus TL, and a systemic administration of free drug (81.0 mg/kg of combretastatin).

RESULTS AND DISCUSSION

In this transplanted tumor model there was no significant increase in the volume of the TL+IR (5 Gy) treated tumors during the initial six days post treatment; all other treatment groups exhibited exponential growth curves after day three. The TL+IR (5 Gy) treatment resulted in a 5.1 day tumor growth delay compared to untreated controls (2). Similar results were obtained using a spontaneous tumor model.

CONCLUSIONS

These findings indicate that preferential targeting of antivascular drugs to irradiated tumors results in significant tumor growth delay. Currently, we are investigating the effects of tumor vascularity on the efficacy of this proposed therapy, using either a MCa-35 or MCa-4 mammary carcinoma tumor models. We would like to know whether highly vascularized tumors (e.g. MCa-35) are more receptive to therapy than more hypoxic tumors (e.g. MCa-4).

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NANOSTRUCTURES OF SHED KING COBRA SKIN AND PERMEATION OF PARABENS

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ABSTRACT

INTRODUCTION

Difficulties in obtaining and using human skin have tempted many workers to employ animal membranes for percutaneous absorption studies. The use of shed snake skin, a non-living tissue which lacks in hair follicles, for permeation studies was reviewed [1]. Shed skins of *Elaphe obsolete* (black rat snake) were reported for use as a model membrane for transdermal research [2, 3]. *Python reticulates*, *Naja Najas Khoatia* and *Ophiophagus hannah* were the other types attempted [4, 5]. Scales of shed skin of *Ophiophagus hannah* (king cobra) were used to investigate *in vitro* permeation of methyl and propyl parabens and the nanostructure of the scales was observed by using atomic force microscopy.

EXPERIMENTAL METHODS

Permeation of methyl, propyl and butyl parabens from saturated buffered solutions (pH 5.5) through a fully hydrated scale of shed king cobra skin into a buffer solution (pH 7.4) was performed using diffusion cells as previously reported [5]. Atomic force microscope (AFM, Seiko SPI400) was employed to investigate the nanostructures of the fully-hydrated scales of the shed skin.

RESULTS AND DISCUSSION

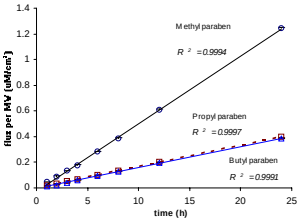

FIG 1 Permeation of methyl( ) propyl ( ) and butyl paraben ( )

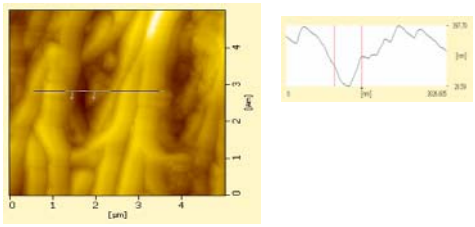

FIG 2 AFM of channels of fully hydrated scale of king cobra (Insert illustrates the 2D scanned along the line)

The mean fluxes of methyl paraben (MW 152.15), propyl paraben (MW 180.20) and butyl paraben (MW 194.23) are 52.6, 16.4 and 16.4 $nM \cdot cm^{-2}\ h^{-1}$ respectively, as shown in Fig 1 Nanostructures of the scales composed of mainly microfibrils and some micropores; the microfibrils being about 500 nm in diameter of the cross-section of the micropores, as indicated by arrows in Fig 2, being upto 500 nm wide and 200 nm deep. These were about 2-10 times larger than those non-hydrated scales of 3 types of snakes[6]. At a fully hydrated state, microfibrils, whose major components were keratins, might swell and the channels of could have been changed to facilitate the permeation.

CONCLUSIONS

Methyl paraben permeated through the scales of shed king cobra at different extent to propyl and butyl parabens, the latters being similar in size. Selectivity of molecular size could be the channel of transport of these paraben derivatives. . The effect of esterase enzyme remained in the shed skin was another factors aware. Channels of potential permeation of the nanostructures, including an ordered microfibrillar arrays and nanopores, would be discussed.

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CHARACTERIZATION OF NANOPARTICLES FOR POROSITY AND FRACTAL DIMENSION

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INTRODUCTION

Although lot of research is being directed in the area of particulate drug delivery, reports on nanoparticle (NPs) porosity and microstructure are still lacking. These two parameters can have a profound effect on the polymer degradation and drug release rate. Similarly, fractal dimension (D) can shed some light on the surface morphology and internal pore structure. Gas adsorption technique is widely used to study the porosity and microstructure of zeolites. We have recently shown that formulation factors affect internal structure of the NPs and thus, the release kinetics of the encapsulated drug[1]. Also, D can serve as a useful tool for explaining drug release kinetics from NPs. This is the first study that reports effect of polymer type and solvent evaporation rate on the porosity and D of NPs.

EXPERIMENTAL METHODS

Blank NPs were prepared by emulsion-solvent evaporation method using different polymers such as PLA, multiblock copolymer of polyethylene glycol (PEG) and PLA {Multi-(PEG-PLA-PEG)}, PEG-*g*-PLA with 1 and 5 mole % grafting densities of PEG (PEG1%-*g*-PLA and PEG5%-*g*-PLA, respectively). The solvent from NP suspension was evaporated under vacuum using magnetic stirrer or rotary evaporator. Drug-loaded particles were prepared using propafenone hydrochloride as a model drug. NPs were characterized for loading efficiency and particle size distribution. Effect of polymer type, solvent evaporation rate and drug loading on porosity of NPs was studied by nitrogen adsorption and parameters like BET surface area, pore size distribution and D were estimated using the Autosorb^{TM} software. Effect of porosity on release will be studied.

RESULTS AND DISCUSSION

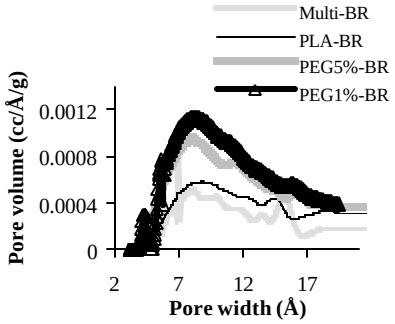
NPs size distribution was unimodal in the range of 180-210 nm. Their pore size distribution showed different pattern with polymer type and solvent evaporation method. NPs of PEG1%-*g*-PLA and PEG5%-*g*-PLA polymers showed clear hysteresis loop in their adsorption isotherms indicating presence of micropores. They also exhibited higher micropore volume and in turn, higher BET surface area (Table 1). Grafted PEG may have increased water influx during NP formation resulting in higher porosity (fig. 1), however, in case of NPs of {Multi-(PEG-PLA-PEG)}; some of the PEG may have been incorporated within the core resulting in the pore blocking effect. Rate of solvent evaporation did not play much role in affecting the internal structure of NPs. D values of all pegylated polymers were higher than PLA suggesting roughness of the surface due to presence of PEG. Effect of porosity and D on drug release kinetics is currently under evaluation.

Table 1: Results of porosimetry of blank NPs

Polymer	Total Pore vol (Vt)*	Micro-pore Vol (Vp)*	BET SA (m^2/g)	Pore width (nm)	D
Multi-(PEG-PLA-PEG)BR	0.09	0.0027	7.76	9.607	2.51
Multi-(PEG-PLA-PEG)BC	0.16	0.0048	13.39	7.892	2.54
PLA-BR	0.22	0.0059	15.86	8.925	2.49
PLA-BC	0.22	0.0057	15.13	9.11	2.49
PEG1%-*g*-PLA -BR	0.30	0.010	28.33	8.025	2.55
PEG1%-*g*-PLA -BC	0.31	0.0099	25.71	7.950	2.55
PEG5%-*g*-PLA -BR	0.13	0.0053	14.45	7.875	2.58

***: cc/ ?/g, B: Blank particles, R: solvent removed on rotavapour, C: solvent removed on magnetic stirrer**

Fig.1: Effect of polymer type on microporosity



From inside to outside: Multiblock, PLA, PEG5%-*g*-PLA, PEG1%-*g*-PLA

CONCLUSION

Porosity of NPs was dependent on the polymer chemistry and amount of PEG. PEG also enhanced surface roughness as seen from D values. Solvent evaporation rate had little effect on any of the parameters. Thus, polymer type and rate of solvent evaporation played a definitive role to affect the microstructure of NPs, which in turn would determine rate of degradation and drug release from NPs.

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TARGETING LIPOSOMES TO THE INFARCTED CARDIAC TISSUE

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INTRODUCTION

Drug targeting to hypoxic areas after an acute myocardial infarction (MI) could provide the means by which proangiogenic/vasculogenic treatments can be selectively targeted to hypoxic tissue.

EXPERIMENTAL METHODS

The Left Anterior Descending coronary Artery (LADA) in the rat animal model is ligated to induce hypoxia in the left ventricle wall (1). Radiolabeled liposomes were allowed to circulate for 24 hours after injection. The "border zone" surrounding the infarct region in the left ventricle wall along with normal myocardium from other parts of the heart were isolated and measured in a scintillation counter for the presence of the radiolabeled liposomes.

RESULTS AND DISCUSSION

Radiolabeled RGD liposomes injected immediately post-MI showed a 60% increase in targeting to infarcted myocardium when compared to adjacent normal myocardium. Radiolabeled RGD liposomes injected 24 hours post-MI showed a 113% increase in targeting to infarcted myocardium when compared to adjacent normal myocardium. There is a significant preferential adhesion of anti-P-selectin coated liposomes (58% and 68%) in the heart immediately and 4 hours post-infarct respectively. We are currently exploring the possibility that the degree of selective targeting may be correlated with the size of the infarct. In our experiments, the targeting to upregulated P-selectin provides the most promising potential target for selectively delivering proangiogenic/vasculogenic compounds to hypoxic areas of the myocardium.

CONCLUSION

There is a significant upregulation of P-selectin due to hypoxia in the heart. This upregulation can be used to target particles to oxygen-deprived parts of the heart *via* ligand coated drug carriers.

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SHAPE AND PH DETERMINE DEGRADATION KINETICS OF PEG-PLA POLYMER NANOCARRIERS

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INTRODUCTION. Loading of potent, but sensitive bio-therapeutics (e.g., catalase) into *polymer nanocarriers* (PNC) can improve drug delivery by protecting cargo from proteolytic inactivation after trafficking into lysosomes (1,2). The loaded drug, catalase, combats vascular oxidative stress, a condition implicated in many disease states including atherosclerosis, thrombosis, hypoxia, hyperoxia, and acute lung injury (3). To enable the proper timing of therapeutic duration and carrier intercellular presence, degradation kinetics of PNCs were evaluated as a function of milieu pH, and tunable design parameters; polymer molecular weight (MW) and PNC size and geometry.

EXPERIMENTAL METHODS. methoxyPEG–poly(lactic acid) (mPEG-PLA) diblock copolymers of various molecular weights (20-71 kDa) were synthesized using a standard ring-opening polymerization (6). *Tube*-shaped and *spherical* PNCs were formulated via a freeze-thaw double emulsion solvent evaporation technique (1). Degradation studies were performed at 37°C in a reciprocal shaking water bath using 3 different pHs (2.5, 5.0, 7.4) to mimic those of relevant circulatory and intercellular compartment environments. PNC size and concentration (dynamic light scattering - DLS), morphology (electron microscopy - TEM), polymer MW and polydispersity (PDI) (gel permeation chromatography - GPC) and degradation byproducts (enzymatic assay) were all monitored over a 30 day period.

RESULTS AND DISCUSSION. PNC synthesized with lower MW polymer possessed an enhanced sensitivity to pH, resulting in accelerated degradation at acidic conditions, and initial particle morphology greatly affected overall PNC stability. *Spherical* PNCs underwent classical bulk degradation with a nominal change in diameter, yet a noticeable decrease in PNC concentration was evident in acidic pH (50 kDa: 30% decrease vs. 20 kDa: 45% decrease, over 20 days). This result indicates that spherical double emulsion PNCs possess a range of stabilities, with some PNC disintegrating more rapidly than others. Alternatively, *Tube*-shaped PNCs experienced a decrease in *measured* diameter (516.4 ± 110 to 312.2 ± 37.2 nm, 2.5pH) as the tubes fractured into smaller segments. In this manner, concentration correspondingly increased as the number of tube fragments in solution increased. There was no measurable change in DLS measurements at neutral pH. Yet, enzymatic assay revealed noticeable erosion in the form of shed end-chain monomers of lactic acid (LA) in 7.4pH (e.g. 65 kDa: $0.2715 \pm 0.34\%_{init}$, t_0 vs. $3.9267 \pm 0.8857\%_{init}$, t_{day28}), while in 2.5pH little LA monomer was detected, suggesting that the majority of degradation was in the form of shed LA oligomers and smaller MW PLA fragments.

CONCLUSIONS. Polyester PNC degradation is dependant upon a complex interplay of size, MW, pH and shape. Overall, strongly acidic environments accelerated degradation, yet there was no significant increase in degradation at lysosomal pH. Bulk degradation effects resulted in a heterogeneous particle loss, with a gradual decrease in the number of *spherical* PNC. *However, tube* PNC fragmentation resulted in an increase in PNC concentration. These differences provide a diverse toolkit for the design of protein therapies, from long-term local depot nanocarriers to single injection PNC formulations.

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LOSS OF ELASTICITY OF AGED HUMAN EPITHELIAL CELLS IN-VITRO AND ITS POSSIBLE RECOVER

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INTRODUCTION

The increase of rigidity of human epithelial tissues with ageing [1] has been implicated in the pathogenesis of many diseases associated with aging including vascular diseases, kidney disease, cataracts, Alzheimer's Dementia [2,3] , complications of diabetes, and cardiomyopathies [4]. It was believed that the increase of tissue rigidity resulted exclusively from cross-polymerization of collagen and elastin in the extra cellular matrix [1]. Many treatments [4,5] for age-related diseases are based on this belief. However, we have recently shown that epithelial cells also become considerably more rigid after aging in vitro[6]. It was also found that the cells had three distinctive regions of different rigidity. We found correlation between the cell rigidity and the density of cytoskeletal fibres. However, it was not clear which type of fibres were measured.

EXPERIMENTAL METHODS

Atomic Force Microscopy (AFM) [6,7] and imunofluorescence have been used in this study to characterize aging human epithelial cells in vitro, both before and after treatment with cytochalasin B.

RESULTS AND DISCUSSION

We found that the fibres correlated with the rigidity are mostly microfilaments, F-actin. Furthermore, using cytochalasin B, a drug that inhibits polymerization of F-actin, we restored the cell rigidities of old cells back to the young level in all three areas of rigidity simultaneously. As described previously, each cell has three distinctive areas of rigidity: nucleus, cytoplasmic, and the edge. The cytoskeletal content is rather different in each area. Therefore, it was not trivial to expect the right amount of decrease to the young level in all three areas simultaneously. Fortunately, the treatment managed to do that. The final rigidity of treated old cells was rather close to the young cells.

CONCLUSION

The results obtained may contribute to a treatment of the age-related loss of elasticity in epithelial tissues, as well as shed light on some aspects of the problem of aging.

ACKNOWLEDGEMENTS

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MAXITARG-A NOVEL TARGETING APPROACH FOR HEPATIC CANCER

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INTRODUCTION AND OBJECTIVE

Targeting anti cancer drugs to cancer cells has become an area of intensive research. 'MAXITARG', an innovative targeting approach deals with the use of a novel-targeting agent (NTA) with high affinity for hepatocellular carcinoma (HCC) cells. Doxorubicin widely used for HCC and well known for its cardiotoxicity and myelosuppression was selected as model drug[1]. The present study discusses the 'MAXITARG' approach in the design of a nanoparticulate drug delivery system of doxorubicin using the NTA, to maximize delivery of doxorubicin to hepatic cells with the following objectives:

- ☞ Preparation of the doxorubicin loaded alginate nanoparticles(NP) without(DoxNP) and with NTA(DoxNTA)
- ☞ Optimization of NP's for high drug loading, encapsulation efficiency and nanosize
- ☞ *In- vivo* evaluation of DoxNP /DoxNTA for targeting efficacy to hepatic cells

EXPERIMENTAL

Preparation of nanoparticles:

DoxNP/DoxNTA were prepared by controlled gelation method.

***In- vitro* evaluation:**

NP's were evaluated for encapsulation efficiency, drug loading and drug content by spectrofluorimetry, particle size distribution using the Coulter N4- Plus (Beckman submicron sizer) and in- vitro drug release. NP's were also characterized by IR spectra, DSC and SEM.

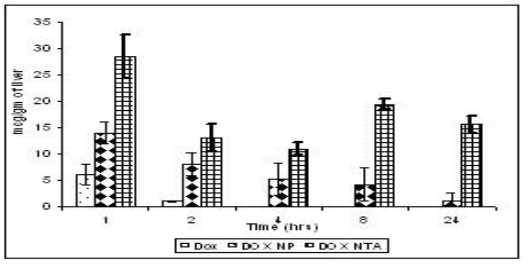
***In- vivo* evaluation:**

DoxNP/DoxNTA/Dox solution (Dox) were injected into the tail vein of rats (10mg/ kg of doxorubicin). At various time intervals blood samples were withdrawn and plasma drug concentration estimated. Subsequently rats were sacrificed, the liver, heart, lung, kidney, spleen excised and analyzed for doxorubicin content by HPLC.

RESULTS AND DISCUSSION

NP's with encapsulation efficiency (> 95%) and nanosize (200-500nm) were optimized. SEM revealed spherical NP with smooth surface. NP's showed sustained in vitro drug release over a period of 60 hrs in pH 7.4 buffer. DoxNTA showed significantly higher uptake of doxorubicin in liver as compared to DoxNP and Dox(fig 1). Besides, DoxNTA showed no detectable drug level in heart. Moreover, Dox and DoxNP showed accumulation of doxorubicin in heart and kidney suggesting possible cardio and renal toxicity.

Fig.1: Doxorubicin concentration in liver.



CONCLUSION

The results suggested maximum hepatic accumulation, minimal cardio and renal toxicity with DoxNTA. 'MAXITARG' therefore represents a promising approach for the treatment of HCC. Moreover Maxitarg could serve as platform technology for hepatic targeting.

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ACKNOWLEDGEMENTS

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TRANSCRIPTIONAL ACTIVATION OF GENE EXPRESSION BY PLURONIC BLOCK COPOLYMERS IN STABLY AND TRANSIENTLY TRANSFECTED CELLS

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INTRODUCTION: Applications of nanotechnology for treatment, diagnosis, monitoring, and control of biological systems has been referred to as "nanomedicine" by the National Institutes of Health. Research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents is at the forefront of projects in nanomedicine. However the effects of the polymeric biomaterials per se on cell and gene expression were not explored. Amphiphilic block copolymers of poly(ethylene oxide) and poly(propylene oxide), Pluronics, enhance gene expression and this enhancement is found to be promoter and strain dependent. In the present work we investigated the effects of Pluronics on gene expression in murine cell models (fibroblasts NIH 3T3, myoblasts C2C12 and mammary adenocarcinoma cells Cl66) transfected with Luciferase and Green Fluorescent Protein (GFP).

EXPERIMENTAL METHODS: NIH3T3, C2C12, and Cl66 cells were stably transfected with reporter genes, luciferase (luc) and Green Fluorescent Protein (GFP). These cells were incubated with Pluronics of various hydrophilic-lipophilic balance (HLB) values for different time points at various concentrations, washed and further incubated for 24 hours. Luciferase and GFP expression was quantified using luminometer and flow cytometry respectively. The cytotoxicity was determined by MTT and Propidium Iodide assays. RT-PCR and Real Time RT-PCR were performed to measure the mRNA levels of the reporter genes. I-κB phosphorylation and NF-κB activation were analyzed using western blot and ELISA methods.

RESULTS AND DISCUSSION: Exposure of the cells to Pluronic resulted in significant enhancement in the expression levels of luciferase and GFP. No cytotoxicity was observed at doses of Pluronics where gene expression was increased. Furthermore, increase in the mRNA levels of luciferase and heat shock protein (hsp68) was also observed, whereas a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was unaffected. Fibroblast and myoblast cells transfected with PathDetect cis-Reporting System constructs were used to examine the involvement of the nuclear factor-κB (NF-κB) and activating protein-1 (AP-1) in Pluronics enhancement. Pluronics enhanced reporter gene expression controlled by NF-κB in both cell models. It also increased expression of a gene under AP-1 in a fibroblast cell line, but not in a myoblast cell line. Activation of the inflammation signaling pathway in myoblast cells by Pluronics was shown by increased I-κB phosphorylation and NF-κB activation.

CONCLUSIONS: Pluronics can enhance the transcription of genes in the cells by activating selected signaling pathways possibly due to mild stress. This effect was found to be promoter selective. In summary, this research suggests that polymers per se can modulate the gene expression and act as 'biological response modulators'. Therefore, the concept of polymers being inert must be revisited.

SEMICONDUCTOR NANOCRYSTALS AND BIOLOGICAL APPLICATION

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Novel strategy for the synthesis of monodisperse nanocrystals was developed. This new method is cheap, reliable, safe and environmentally benign. The nanocrystals synthesized by this new method, including semiconductor nanocrystals (quantum dots) CdS[1], CdSe, CdTe[2], PbSe[3], and magnetic nanocrystals, Fe_3O_4[4] (magnetite), have wider size range, and narrower size distribution (less than 10%). Through this new method, one can control the size, shape, and crystal structure of the aimed nanocrystals by simply changing the ligands used in the synthesis. With the high quality nanocrystals, some basic physical constants, such as extinction coefficients of semiconductor nanocrystals were accurately measured[5]. A simple method was also developed to transfer the above-mentioned organic-media synthesized high quality nanocrystals to aqueous media (pure or buffered water). The water-soluble nanocrystals keep their original properties in organic media. For example, water-soluble semiconductor nanocrystals have the same absorption and emission spectra, the same quantum yield, and the same size and size distribution as the ones dispersed in chloroform. The water-soluble nanocrystals are stable in pure water and conventional biological buffers.

Keywords: semiconductor nanocrystals, magnetic nanocrystals, iron oxide, quantum dot, extinction coefficient.

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HPMA-STABILIZED LONG-CIRCULATING DNA NANOPARTICLES WITH SONOPORATION ENHANCED TRANSFECTION

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INTRODUCTION

Self-assembly DNA nanoparticles based on reducible polycations (RPC) and stabilized by surface coating with multivalent HPMA copolymers exhibit significantly improved plasma circulation and general disposition properties compared with non-stabilized nanoparticles.[1] Biophysical, transfection, and pharmacokinetic properties of HPMA-RPC/DNA nanoparticle was tested in the study. Transient cell membrane permeabilization by ultrasound was successfully used to increase the transfection efficiency of the polyplexes tested and to eliminate the reliance of their activity on chloroquine.

EXPERIMENTAL METHODS

The multivalent HPMA copolymer with $M_w \sim 3.5\times10^4$ was synthesized by copolymerization of HPMA with methacryloylglycylglycine 4-nitro- phenyl ester and contained 8 mol% of the reactive co-monomer. RPC with $M_w \sim 2\times10^5$ was synthesized by DMSO-mediated oxidative polycondensation of $CK_{10}C$ peptide. DNA used in our studies was gWiz™ Luciferase reporter gene expression plasmid purchased from Aldevron. All nanoparticles were prepared at pH 7.0 using RPC or control PLL and N:P ratio of 2. The nanoparticles were coated by adding calculated amount of HPMA copolymers, followed by adjustment of pH to 7.8.

RESULTS AND DISCUSSION

The studies confirmed that using DNA nanoparticles based on reducible polycations leads to a higher transfection activity compared with control nanoparticles based on non-reducible polycations in all cell lines tested. The observed difference in transfection efficiency is assigned to increased intracellular disassembly of HPMA-RPC/DNA nanoparticles. Glutathione is likely to be the major reducing component responsible for intracellular activation of HPMA-RPC/DNA. Ultrasound- mediated effects on the transfection efficiency of HPMA-RPC/DNA were investigated in 4T1 mouse mammary carcinoma cells. As shown in Fig. 1., ultrasound exposure resulted in a 560-fold increase of the gene expression in the case of naked DNA and more than a 110-fold increase in the case of HPMA-RPC/DNA when compared to sham experiments. Although HPMA-RPC/DNA offers no obvious advantage compared with naked DNA in vitro, the favorable pharmacokinetic properties of HPMA-RPC/DNA are expected to bring significant advantages in vivo. Rapid hepatic clearance and metabolic instability of naked DNA in the systemic circulation makes it unsuitable, unlike HPMA-RPC/DNA, for systemic intravenous administration and delivery into distant targets such as tumors.

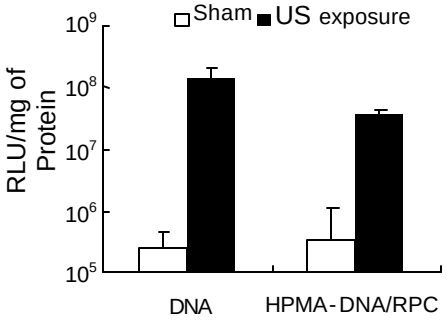

Fig. 1. Effect of ultrasound on transfection activity of HPMA-RPC/DNA nanoparticles. Suspension of 4T1 mouse mammary carcinoma cells with the nanoparticle or naked DNA and ultrasound contrast agent Optison® was exposed to ultrasound (1 min, 0.2 MPa) using a 1.9-cm diameter, air-backed transducer operating at 2.25 MHz. Luciferase activity was measured 48 h after the exposure. (n =5)

CONCLUSION

Our results show that the HPMA-RPC/DNA nanoparticles exhibit favorable disposition profile after intravenous administration and promising transfection activity in vitro. In addition, the HPMA-RPC/DNA nanoparticles are compatible with both molecular targeting using protein ligands as well as physical targeting using ultrasound-directed cavitation. As such, these gene delivery vectors have the potential to permit efficient systemic delivery of therapeutic genes targeted by local ultrasound treatment.

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ASSEMBLY OF HYDROGELS WITH CONTROLLED PROTEIN - DELIVERY PROFILES VIA THE USE OF PEPTIDE - POLYSACCHARIDE INTERACTIONS

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INTRODUCTION

Hydrogels that are responsive to the biological environment would find widespread use in drug delivery. In a critically important biological environment, the extra-cellular matrix (ECM), proteoglycans play numerous roles via their interactions with other proteins. Accordingly, these protein-polysaccharide interactions may also be useful to direct the assembly of hydrogels, and to control the mechanical response and biological properties of those hydrogels. We have employed noncovalent interactions between heparin and a heparin-binding, coiled-coil peptide (PF4[1]) to mediate network assembly. This peptide mimics the heparin-binding domain of human platelet factor 4. Both the peptide and low molecular weight heparin (LMWH) were attached to the termini of four-arm star poly (ethylene glycol) (PEG) and incorporated into hydrogel systems via the interactions between PF4 and LMWH. Controlled protein delivery from these assembled hydrogels has been demonstrated.

EXPERIMENTAL METHODS

The synthesis of the PEG-LMWH conjugate exploited in hydrogel assembly was conducted via Michael-type addition reactions between maleimide-functionalized heparin and thiol-terminated four-arm star PEG, as previously reported.[2] The PF4 peptide was prepared on Rink Amide MBHA resin via solid phase peptide synthesis with Fmoc-protection using a PTI PS3 peptide synthesizer (Protein Technologies, Inc.) and purified via preparative-scale, reverse-phase HPLC (Delta 600 HPLC, Waters). The synthesis of the PEG-PF4 conjugate was conducted via addition reactions between vinyl sulfone terminated four-arm PEG and cysteine-terminated PF4 peptide.[2] PEG-LMWH/PEG-PF4 hydrogels were formed via adding 5 wt% solutions of PEG-PF4 to 2.5 wt% solutions of PEG-LMWH in PBS, to afford hydrogel networks. The rheological properties of these networks were determined with a stress-controlled Rheometrics Paar Physica rheometer, via methods as previously described.[3] bFGF release and hydrogel erosion experiments were performed at 4°C in 24-well polystyrene assay plates (Corning Inc.), following protocols previously reported.[2] The amount of bFGF in each sample was measured with a bFGF Quantikine kit (R&D Systems). Hydrogel erosion was quantified via a combination of fluorimetric and gravimetric assays.

RESULTS AND DISCUSSION

The PF4 peptide has been shown to have high heparin-binding affinity and fast binding kinetics via heparin-sepharose chromatography and surface plasmon resonance (SPR) experiments, respectively. Rheological characterization of assembled hydrogels verified that the hydrogels exhibit elastic behavior that arises from association of PEG-LMWH and that the hydrogel elastic moduli increase with addition of PEG-PF4. bFGF is released slightly more slowly from the PEG-LMWH/PEG-PF4 hydrogels than from the PEG-LMWH hydrogels. Erosion profiles of these hydrogels demonstrate that the erosion of the PEG-LMWH hydrogels is faster than the erosion of the PEG-LMWH/PEG-PF4 hydrogels. Based on these and previously reported results,[2] the bFGF release from the noncovalently assembled hydrogels is suggested to be mainly an erosion-controlled process that can be manipulated via appropriate choice of polysaccharide-peptide interactions.

CONCLUSIONS

The process of growth factor bFGF release from PEG-LMWH/PEG-PF4 hydrogels is mainly through an erosion-controlled pattern. Hydrogels with engineered mechanical properties and biological activities based on peptide-polysaccharide interactions may find their use in controlled delivery of therapeutics and in other biological applications.

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Author Index

Agnew, H. 29
Allen, C. 91,92
Allen, T. 49
Allen, S. 23
Al-Zubaidi, E. 66
Avnir, Y. 67
Bachelder, E. 88
Bailey, R. 29
Barenholz, Y. 67
Bentley, W. 81
Berk, E. 94
Betigery, S. 27
Betka, J. 68
Bogdanov, A. 41
Boucek, J. 68
Bronich, T. 51
Brown, D. 96,99
Bulger, E. 78
Bunimovich, Y. 29
Burger, A. 69
Chauhan, A. 37
Cheng, J. 70
Cheng, X. 71
Chilkoti, A. 53
Chitropas, P. 97
Chong, P. 96,99
Chytil, P. 72,79
Cislerova, L. 85
Colon, F. 100
Colvin, V. 104
Cuchelkar, V. 47
Cui, X. 94
Dadashzadeh, S 73
Davies, M. 23
Derakhshandeh, K. 73
DeShong, P. 39
DeSimone, J. 80
Devarajan, P. 74,102
Ding, H. 47
Discher, D. 100
Doble, M. 74
Dowling, M. 75
Duncan, R. 57
Dziubla, T. 76,94,100
Ehrman, S. 89
Elbayoumi, T. 77
Elizarov, A. 29
El-Sayed, M. 31,78
English, D. 39,89
Etrych, T. 72,79
Euliss, L. 80
Farokhzad, O. 70
Fernandes, R. 81
Fiebig, H. 69
Furst, E. 106
Geng, Y. 100
Ghandehari, H. 43,83,84,87,93
Gupta, B. 82
Gupta, Y. 86
Haider, M. 83
Hatefi, A. 84
Heath, J. 29
Hildgen, P. 98
Hochhaus, G. 73
Hoffman, A. 31,78
Hovorka, O. 85
Huesca, M. 91
Isaacs, L. 89
Iyer, S. 101
Jain, A. 86
Jain, N. 86
Jain, S. 86
Johns, R. 31
Kabanov, A. 61,103
Kataoka, K. 59
Kawazu, T. 95
Khademhosseini, A 70
Khamlert, C. 97
Khan, M. 63
Khare, P. 86
Kiani, M. 96,99
Kiick, K. 25,106
Kitchens, K. 87
Kizelsztein, P. 67
Klausner, E. 88
Koh, I. 89
Kolb, H. 29
Konak, C. 72
Koo, O. 55
Kopecek, J. 47
Kopeckova, P. 47
Krishnadas, A. 55
Kuhn, L. 71
Kwong, G. 29
Laginha, K. 49
Langer, R. 70

Lee, J. 75
Lee, S. 35
Leferovich, J. 94
Leng, Q. 90
Leong, K. 83,88
Levchenko, T. 82
Levy -Nissenbaum, E. 70
Line, B. 43,93
Liu, J. 91,92
Lovett, A. 39
Malugin, A. 47
Manickam, D. 105
Matzinger, P. 88
Maynor, B. 80
Miller, D. 105
Minko, T. 27
Mitra, A. 43,93
Mixson, A. 90
Mongayt, D. 82
Mrkvan, T. 79
Muro, S. 94
Muzykantov, V. 76,94,100
Nagasaki, T. 95
Nan, A. 43,93
Naparstek, Y. 67
Nori, A. 47
Onyuksel, H. 55
Oupicky, D. 105
Pakunlu, R. 27
Papadimitriou, J. 93
Park, J. 39
Pastorino, F. 49
Pattillo, C. 96,99
Payne, G. 75
Plocova, D. 68
Pongjanyakul, T. 97
Ponzoni, M. 49
Prakash, A. 39
Pratontep, S. 97
Priprem, A. 97
Qiu, W. 94
Raghavan, S. 75
Reyna, L. 37
Rihova, B. 68,79,85
Roberts, C. 23
Rolland, J. 80
Rubinstein, I. 55
Rungsardthong, U. 97
Sadoqi, M. 45
Sant, S. 98
Sapra, P. 49
Sausville, E. 69
Schuler, J. 69
Scott, R. 96,99
Sherifi, I. 70
Shinkai, S. 95
Shuvaev, V. 76
Simone, E. 100
Sokolov, I. 101
Sonavane, G. 74,102
Soni, V. 86
Sriadibhatla, S. 103
Stayton, P. 31,78
Strohalm, J. 68,85
Subr, V. 68,85
Svenson, S. 37
Swaan, P. 87
Szoka, F. 21
Tender, S. 23
Teply, B. 70
Tliba, S. 76
Tomalia, D. 37
Torchillin, V. 77,82
Ulbrich, K. 68,72,79,85
Ulmansky, R. 67
Varughese, B. 89
Wang, B. 96,99
Wang, X. 89
Wang, Y. 27
Welch, C. 80
Williams, P. 23
Woodle, M. 33
Woodworth, C. 101
Yang, Z. 103
Yeo, W. 29
Yu, W. 104
Zachariah, M. 39
Zeng, F. 92
Zhang, L. 106
Zhou, Q. 105

Notes

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Notes

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Notes