SYMPOSIUM I

I: Biomaterials for Drug Delivery

December 1 - 2, 2003

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* Invited paper

SESSION I1: Hydrogels
Chair: Ashutosh Chilkoti
Monday Morning, December 1, 2003
Republic A (Sheraton)

8:45 AM *I1.1
Molecular Design and Cellular Response of Novel Intelligent Mucoadhesive Carriers for Oral Delivery of Proteins and Chemotherapeutic Agents. Nicholas A Peppas[1,2], James O Blanchette[2] and Kristy M Wood[2]; [1]Chemical Engineering, University of Texas, Austin, Texas; [2]Biomedical Engineering, University of Texas, Austin, Texas.

Complexation graft copolymers of poly(methacrylic acid-g-ethylene glycol), designated as P(MAA-g-EG), have been shown to be effective in oral delivery of proteins and chemotherapeutic agents. Their hydrogen bonding complexation/decomplexation characteristics render these responsive hydrogels able to protect the agent from the harsh, acidic environment of the stomach before releasing the bioactive agent in the small intestine. Studies have focused on oral administration of insulin, calcitonin and bleomycin through release and absorption in the upper small intestine, the duodenum. Proteins, such as insulin, have been successfully loaded into the P(MAA-g-EG) hydrogels through imbibition whereas bleomycin has been loaded by in situ polymerization. The ability of the carriers to release the agents in response to a pH increase designed to mimic the passage from the stomach to the duodenum has been evaluated. The swelling of the carrier as a result of the change in complexation state caused by the shift in the environmental pH (from 2.0 to 7.0) led to increased release of the agents at the higher pH simulating the environment of the duodenum. Studies of cell-polymer interactions have shown that the carriers can inhibit the activity of Ca2+ dependent proteolytic enzymes, increase the residence time of the carrier in the small intestine by mucoadhesion, and reversibly open the tight junctions between the intestinal cells. Transport studies using Caco-2 cells, a widely used in vitro model for intestinal absorption of drugs, have proven the efficacy of the polymeric system. The opening of the tight junctions was measured by measuring the transepithelial electrical resistance (TEER) of the cell monolayer. Synergic effect of the solutes and the complexation hydrogels on the cell monolayer was studied by measuring the reduction in TEER values of the cell monolayers. Cytotoxicity analysis has shown the biocompatibility of the P(MAA-g-EG) carriers as well.

9:15 AM I1.2
Formation, Erosion, and Drug Release from Hydrogel Nanoparticle Aggregates Composed of pHEMA and pHEMA-MAA. John Vincent St. John, Daniel G Moro and Bill Ponder; Hydrogel, Access Pharmaceuticals, Dallas, Texas.

Hydrogel matrices have a long history of application as reservoirs of active drugs. However, it can be difficult to achieve uniform incorporation and controlled release of macromolecules from hydrogels. Access Pharmaceuticals has developed a hydrogel-based drug delivery system composed of poly-(2-hydroxyethylmethacrylate) (pHEMA) nanoparticles and copoly-(2-hydroxyethylmethacrylate-methacrylic acid) (pHEMA-MAA) nanoparticles that allows controlled incorporation and release of macromolecules. Nearly monodisperse particles are produced with diameters ranging from 30 to 800 nm. Particles are precipitated from solution to form a solid, non-covalently linked aggregate of particles. Aggregates composed of pure pHEMA particles will not redisperse into a solution of particles. Incorporation of methacrylic acid as a comonomer results in aggregates that redisperse at physiological pH. Aggregates have many of the properties of bulk pHEMA; however, unlike a bulk network, they can be molded or extruded. An important property of these aggregates results from the packing of nanoparticles into a condensed solid. Spherical particles pack with interstitial spaces, and variation of particle size within the aggregate results in larger or smaller spaces and channels. Macromolecules (*e.g.* proteins) can be trapped in the aggregate during precipitation. Protein diffusion rate is controlled by altering the nanoparticle size with accompanying control of channel size. The dispersion rate of pHEMA-MAA aggregates allows further control over macromolecule release rates. The dispersion rate of an aggregate is controlled by varying the ratio of HEMA to methacrylic acid in the particles. Increased methacrylic acid content results in aggregates which disperse at a faster rate. Molecules trapped between particles are released as the aggregate falls apart. Labeled proteins or dextran markers have been studied to determine the effect of nanoparticle size, and/or methacrylic acid composition on macromolecule release rates. Release profiles for molecules with molecular weights ranging from 12 kDa to 2000 kDa have been characterized. These results demonstrate that hydrogel aggregates warrant further investigation as drug delivery devices.

9:30 AM I1.3
Design of Hydrogel-Based Antigen Delivery Particles for Dendritic Cell Activation. Siddhartha Jain[1] and Darrell J Irvine[1,2]; [1]Biological Engineering, MIT, Cambridge, Massachusetts; [2]Department of Material Science and Engineering, MIT, Cambridge, Massachusetts.

Dendritic cells (DCs) play a crucial role in the generation of primary immune responses. DCs engulf antigen through receptor-mediated endocytosis, micropinocytosis, or phagocytosis, and process it into antigenic peptides within the endosomes and in the cytosol. In robust immune responses, DCs are activated by inflammatory signals, which cause them to export these peptides antigens to their surface in the cleft of Major Histocompatibility Complex (MHC) molecules and upregulate costimulatory receptors required for activation of T cells. Mature DCs migrate to secondary lymphoid organs, where they activate naïve T cells. We seek to develop a vaccine that triggers this complete sequence of cellular/ molecular events believed to be critical for initiation of an effective immune response and induction of protective immunity. We are developing virus-mimetic hydrogel microparticles for simultaneous antigen-delivery and DC maturation at a peripheral tissue in vivo. Our initial studies have focused on 1) the synthesis of hydrogel microparticles for efficient loading of antigen, 2) conjugation of selected maturation and cross presentation-inducing agents on the particle surface to obtain an effective cytotoxic T-lymphocyte (CTL) response, and 3) studying DC response to these particles upon uptake in culture in vitro. We have synthesized PEG-based particles using salting-out emulsion polymerization modified for encapsulation of a model antigen protein (ovalbumin); these particles have a high protein loading of 730μg protein /mg particles and overall efficiency of encapsulation of 50%. We are currently studying the effect of surface-immobilized maturation- and cross presentation- inducing factors including unmethylated CpG oligonucleotides, anti-CD16/CD32, and CD40 ligand on presentation on DC activation. This vaccine strategy may be of general importance for the prevention and/or treatment of various diseases including cancer, HIV, influenza, and hepatitis C.

9:45 AM I1.4
Progesterone Release from Intrafallopian Tube Gelling Materials. Brent Vernon[1], Frank Fusaro[1], Brad Borden[1] and Kelly H Roy[2]; [1]the Harrington Department of Bioengineering, Arizona State University, Tempe, Arizona; [2]Obstetrics and Gynecology, Banner Good Samaritan Hospital, Phoenix, Arizona.

We are investigating the use of in situ gelling materials based on the Michael-type reaction between pentaerythritol tetrakis (3 mercaptopropionate) (QT) and either poly(ethylene glycol) diacrylate (PEGDA) or poly(propylene glycol) (PPODA) diacrylate for permanent contraception by fallopian tube embolization. Presence of the material in the fallopian tube acts to inhibit conception and the progesterone can provide an additional chemical deterrent to pregnancy. The progesterone would also decrease the bleeding and cramping often associated with the presence of the device within the fallopian tubes. For the PEGDA samples, 33 mg or 165 mg of progesterone was combined with 288 mg of PEGDA (525 g/mol) and 122 mg of QT. The resulting mixture was vortexed for 90 seconds. Following the last vortex, 136.7 mg of PBS (pH 7.35) was then added and the solution again vortexed for 2 min. The mixture was injected into tygon tubing having an i.d. of 0.9 mm, allowed to cure overnight and cut into 1 cm long samples. The drug loading was confirmed after the release experiments by extracting the remaining progesterone in 10 mL of methanol for 2 weeks and detecting the drug at 247 nm. The same procedures were used for PPODA (575 g/mol) with 32 mg or 160 mg of progesterone combined with 270 mg of PPODA, 122 mg of QT, and 130.7 mg of PBS (pH 12). All the drug loaded samples (3 each for PEGDA and PPODA at each drug loading) were placed individually into labeled 50 ml centrifuge tubes. PBS (50 ml, pH 7.35) was added to each tube. Tubes were capped and placed inside a rocker-incubator set at 37°C, 75 rpm. The drug concentration in solution was determined by UV absorbance at 247 nm. SEM was used to evaluate the progesterone particles on the surface of the PPODA samples. The samples showed fluctuating release during the first hours and then steady release for a prolonged period (time dependent on the mass of drug loaded). Results revealed zero-order progesterone release. Rates were approximately 40 μg per day from 0.9 mm by 1 cm tubules for both PEGDA and PPODA gel systems. The drug loading, ~5-wt% to ~25-wt%, had minimal effect on the steady state release rate from these systems. There was an only marginal increase in the steady state release rate in the PEGDA systems compared to the PPODA systems, despite the large difference in gel hydrophobicity and in gel morphology. This work demonstrates that these in situ-gelling materials based on the Michael-type reaction between thiol functional groups and acrylate function groups can be used to provide prolonged release of progesterone upon in situ gelation in the fallopian tube.

10:30 AM I1.5
Swelling Behavior of Polyelectrolyte Hydrogels for Drug Delivery Applications. Jamie L Ostroha[1], Mona Pong[3], Nily Dan[2] and Tony Lowman[2]; [1]Materials Science and Engineering Department, Drexel University, Philadelphia, Pennsylvania; [2]Chemical Engineering Department, Drexel University, Philadelphia, Pennsylvania; [3]School of Biomedical Engineering, Drexel University, Philadelphia, Pennsylvania.

Hydrogels are three dimensional, water-swollen networks that are insoluble in water because of crosslinks. They have been studied extensively for biomedical applications such as drug delivery devices. Polyelectrolyte hydrogels are polymer networks that contain either acidic or basic pendant groups. Polyelectrolyte hydrogels are extremely responsive to changes in environmental pH and ionic concentration, and the swelling forces that develop are larger than those found in neutral hydrogels. This environmentally responsive behavior makes polyelectrolyte hydrogels good candidates for drug delivery applications since in addition to their high water content and rubbery nature, they can be made to behave differently in different areas of the body. In this paper, the swelling behavior of methyl acrylic acid anionic hydrogels is characterized theoretically and experimentally. Specifically, we focused on the effect of the molecular weight between crosslinks and addition of pendant, polyethylene glycol grafts on the swelling behavior. We have shown that polyelectrolyte hydrogels exhibit a significant swelling response not only to changes in pH but also to ionic concentration. The mathematical model developed is capable of accurately predicting the swelling behavior of these polyelectrolyte hydrogels. This model takes into consideration charged groups on the polymer chains, molecular weight between crosslinks, PEG pendant groups, and changes in environmental conditions (pH and ionic concentration). The collapsed to swollen transition region can be controlled both in its width and the pH at which this region occurs.

10:45 AM I1.6
The Use of Combinatorial Chemistry in the Development of Temperature Sensitive Hydrogels. Elena Garreta, Alex Marin, Jose Ignacio Borrell, Carles Colominas and Salvador Borros; Molecular Engineering Group, Institut Quimic de Sarria-Universitat Ramon llull, Barcelona, Spain.

Recently, researchers have paid considerable attention on hydrogels that change their structure and properties in response to external stimuli, due to its potential use as biomaterials for drug delivery and tissue regeneration. Many kinds of stimuli are available, such as temperature, pH, electric field, pressure. Particularly, temperature sensitive hydrogels are very interesting for the development of injectable scaffolds for tissue engineering applications (1-4). Thermosensitive hydrogels dramatically change their swelling degree in water above or bellow a certain temperature, called lower critical solution temperature (LCST) or upper critical solution temperature (UCST), respectively (2). The temperature dependence of certain molecular interactions, such as hydrogen bonding and hydrophobic effects, contribute to this type of phase separation (5). For drug release applications, mainly LCST systems are relevant. The phase behavior of these hydrogels can be manipulated by the addition of more hydrophilic or more hydrophobic monomers. Numerous studies have been made to understand the mechanisms of temperature induced phase separation and the influence of co-monomers on the LCST (6, 7). In the present work, a combinatorial chemistry method to synthesize a diversity of temperature responsive hydrogels has been developed. Different acrylamide and acrylic acid derivatives have been used as monomers and bisacrylamide derivatives as crosslinkers. The crosslinking reactions have been carried out with the addition of ammonium peroxydisulfate (AP) as the initiator and N,N,N,N?-tetramethylethylenediamine (TEMED) as the accelerator. The type of monomers and crosslinkers, their concentration and the reaction temperature have been established as diversity factors. The resultant hydrogels have been characterized in terms of LCST determination by a spectrophotometric method and DSC. The aforementioned combinatorial approach has allowed us to study a wide range of temperature sensitive hydrogels on an easier and relatively faster way than traditional approaches. Furthermore, the complete development of a library of temperature responsive hydrogels should allow us to have a set of gels for a wide range of possible applications. (1) T. Dobashi, T. Narita, J. Masuda, K. Makino, T. Mogi, H. Ohshima, M. Takenaka, B. Chu, Langmuir 14 (1998) 745 (2) L. E. Bromberg, E. S. Ron, Advanced Drug Delivery Reviews 31 (1998) 197-221 (3) R. A. Stile, K. E. Healy, Biomacromolecules 2 (2001) 185-194 (4) R. Landers, U. Huebner, R. Schmelzeisen, R. Muelhaupt, Biomaterials 23 (2002) 4437-4447 (5) R. A. Stile, W. R. Burghardt, K. E. Healy, Macromolecules 32 (1999) 7370-7379 (6) H. Feil, Y. H. Bae, J. Feijen, S. W. Kim, Macromolecules 26 (1993) 2496-2500 (7) Y. Kaneko, S. Nakamura, K. Sakai, A. Kikuchi, T. Aoyagi, Y. Sakurai, T. Okano, Journal of Biomaterials Science Polymer Edition, vol. 10, No. 11 (1999) 1079-1091

11:00 AM I1.7
A Candidate for Smart Drug Delivery: Novel Supramolecular Hydrogels Response to A Ligand-Receptor Interaction. Yan Zhang[1], Bengang Xing[1], P. L. Ho[2], Hongwei Gu[1], Zhimou Yang[1] and Bing Xu[1]; [1]Chemistry, Hong Kong University of Science & Technology, Hong Kong, NA, Hong Kong; [2]Microbiology, University of Hong Kong, Pokfulam Road.

Here we report the first hydrogel formed via self-assembly of a potent antibiotic molecule–vancomycin-pyrene (**1**)–into a helical polymer that gels in water. Via hydrophobic interaction and hydrogen bonding, molecules of **1** self-assemble into polymer-like chains in water; these chains aggregate into fibers that entangle into networks to form a hydrogel. The high-resolution transmission electron micrograph (TEM) shows the individual self-assembled polymer chains in the hydrogel. In addition, vancomycin-pyrene (**1**) itself also inhibits vancomycin resistance enterococci (VRE). We envision that such an approach will contribute to the fundamental knowledge of hydrophobic interaction and hydrogen bonding and promise new type of biomaterials. We will also describe novel supramolecular hydrogels formed by N-(Fluorenyl-9-Methoxycarbonyl) dipeptides via hydrogen bonding and hydrophobic interactions. These hydrogels exhibit gel-sol phase transition not only by thermal or PH perturbation, but also by a specific ligand-receptor interaction. The ability to control the properties of supramolecular hydrogels via a specific ligand-receptor interaction may ultimately lead to a disease or tissue-specific drug delivery system.

11:15 AM I1.8
Effect of Polymer Composition on the Gelation and Release Behavior of PLGA-g-PEG Biodegradable Thermoreversible Gels. Barbara J Tarasevich[1], Anna Gutowska[1], Xiaohong Li[1] and Sreedhara Alavattam[2]; [1]Materials Science, Pacific Northwest National Laboratory, Richland, Washington; [2]Biotechnology, Battelle Memorial Institute, Columbus, Ohio.

Graft copolymers consisting of a poly(DL-lactic acid-co-glycolic acid) backbone grafted with polyethyelene glycol (PLGA-g-PEG) side chains were synthesized and formed thermoreversible gels in aqueous solutions which exhibited solution behavior at low temperature and sol-gel transitions at higher temperature. Synthesis parameters such as feed ratio and temperature affected the degree of incorporation of PEG chains and the resulting polymer composition and the polymer molecular weight. The gelation temperature could be systematically controlled by varying the amount of PEG grafting with increasing PEG composition resulting in increasing gelation temperature. This resulted in low viscosity solutions which were injectable at room temperature and formed gels at 37 degrees C. In-vitro studies demonstrated the controlled release of peptides over one to two week time periods.

11:30 AM I1.9
Synthesis, Characterization and Hydrocortisone Release from Poly (DL-lactide-co-glycolide)/ Polyvinyl Alcohol Composite. Xinyin Liu and Anthony M. Lowman; Chemical Engineering, Drexel University, Philadelphia, Pennsylvania.

In this research, a polymeric composite drug delivery system was developed for sustained release of an anti-inflammatory hormone, hydrocortisone. Poly (DL-lactide-co-glycolide) (PLGA) microparticles with sizes of 5-10 μm were fabricated using single emulsion method, and then incorporated into physically crosslinked polyvinyl alcohol (PVA) hydrogels through freeze-thawing method. The composite structure was maintained with PVA crystallites serving as crosslinking points, which were verified using differential scanning calorimetry (DSC). Notably, the melting peak of PVA was shifted to lower temperature due to the addition of PLGA. FTIR spectra also confirmed the presence of crystallites which had an IR peak at 1142 cm^{-1}, while the peak height decreased with the presence of PLGA in the composite. The surface morphology change of the composite upon degradation in PBS solution (pH 7.4) was studied using environmental electron scanning microscopy (ESEM). The in vitro release of hydrocortisone was preformed in dissolution cells in PBS (pH 7.4) at 37°C under sink conditions for one month. Sustained release with lowered initial burst effect was obtained using the PLGA-PVA composite, comparing to the fast release from PVA hydrogel or the high initial burst release from PLGA microparticles, respectively. Furthermore, a 'sandwich' composite was prepared by adding a layer of PVA on both sides of the composite described above. Similar trend was obtained for the release of incorporated hydrocortisone, with further depressed initial burst effect during the early stage of the release. The three layers of the composite were well attached to each other with no separation observed during the dissolution studies. The composites can be further applied to tissue engineering associated with drug delivery since a porous scaffold can be formed after the degradation of PLGA microparticles. Therefore, it

is a potential candidate as implantable medical devices for controlled and targeted drug delivery.

SESSION I2: Nanoparticles and Nanocomposites
Chair: Dan Luo
Monday Afternoon, December 1, 2003
Republic A (Sheraton)

1:30 PM I2.1
Immunologically Targeted Rhenium Oxide Nanoparticles for the Radiotherapy of Solid Tumors. Brian W Smith[1], David E Luzzi[1] and Gregory P Adams[2]; [1]Materials Science and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania; [2]Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania.

Significant success has been realized in treating hematological malignancies with antibody-conjugated radioisotopes, or radioimmunotherapy (RAIT). However, RAIT has proved less effective in treating solid tumors. We hypothesize that it will be possible to improve the efficacy of RAIT in this latter setting by packaging radioisotopes in the form of nanoparticles that could each deliver multiple cytotoxic events. We have selected isotopes of the radiometal rhenium as they can readily form nanoparticles, and two have physical decay properties that have been associated with effective RAIT. Using the stable form of rhenium, we have succeeded in synthesizing populations of 1-100 nm diameter rhenium oxide nanoparticles via a method compatible with the production and administration of a radiopharmaceutical. Nanoparticle modification with surface functional groups and characterization by analytical transmission electron microscopy and dynamic light scattering will be discussed. We will describe our work to date on conjugating the nanoparticles with single-chain Fv (scFv) antibody fragments against tumor-specific parenchymal and vascular antigens. These preliminary results indicate that we are now in position to create the first viable nanoparticle radioimmunoconjugate. Such molecules could greatly increase the therapeutic efficacy of RAIT for the treatment of solid malignancies.

1:45 PM I2.2
Development Of Dendrimer-Gold Radioactive Nanocomposites To Treat Cancer Microvasculature. Lajos Peter Balogh[1,4,5], Shraddha S Nigavekar[2], Leah Minc[3], Philip A Simpson[3], Lok Yun Sung[2], Xiangyang Shi[1], Andrew T Cook[3] and Mohamed K Khan[2]; [1]Internal Medicine, University of Michigan, Ann Arbor, Michigan; [2]Radiation Oncology, University of Michigan, Ann Arbor, Michigan; [3]Michigan Memorial Phoenix Project, University of Michigan, Ann Arbor, Michigan; [4]Biomedical Engineering, University of Michigan, Ann Arbor, Michigan; [5]Macromolecular Science and Engineering, University of Michigan, Ann Arbor, Michigan.

Our goal is to exploit the differences between the normal and tumor microvasculature to deliver therapeutic nanoparticles specifically through the tumor microvasculature to the tumor (using size and charge characteristics of the particles), or to the tumor microvasculature itself directly (using surface recognition). This is a general approach, because all tumors must build a microvasculature in order to grow. We are presently focusing on the development of a multifunctional nanodevice platform based on dendrimer nanocomposites to treat cancer. We have a particular interest in particles that permit localization to tumors or other specific organs following systemic administration. As an example, the fabrication and utility of gold nanocomposite devices (NCDs) to selectively deliver radiation to tumors by exploiting differences between normal and tumor vasculature will be described. Water-soluble NCDs are synthesized as stable hybrid nanoparticles composed of guests and spherical poly(amidoamine) dendrimer hosts. These NCDs can have a specific charge, content and adherence to cells and therefore may also be used to map certain cell compartments and structures, making both imaging and therapy possible. We focus on the development of {Au-198} nanocomposites to deliver beta-radiation to tumors in a B16 melanoma bearing mice model. The radioisotope containing nanodevices have a well-defined and variable size (in a range of 5-75 nm), and a specific surface to permit both size related and/or surface recognition targeting of tumor tissues and cells. It is easy to observe and identify gold NCD particles in tissues or cells, since no other objects can be found in tissues or cells that are similar in shape, size and contrast. Activation of gold atoms in the composite nanoparticle is achieved by direct irradiation in a neutron beam making both imaging and radiotherapy possible. Biodistribution experiments were performed using H-3 labeled dendrimer hosts that have long half-life (11 yrs) and non-radioactive (cold) Au isotopes having low safety risks for the experimental procedures. The amount of NCDs in tissue samples was determined through instrumental neutron activation analysis of their gold content. Delivery of {198-Au} nanocomposites to tumors was performed on various mouse tumor models and various imaging techniques to demonstrate the feasibility of the general nano-device concept in actual carcinomas in vivo. This technique enables us to first synthesize the nanomaterials, then fabricate and characterize the complex device, and finally activate and use the NCDs. This approach will considerably shorten the development of new active NCD systems allowing the use of other short half-life radionuclides.

2:00 PM I2.3
Controlled Delivery of Oncology Drugs using Silica Nanoparticles. Christophe J Barbe[1], Linggen Kong[1], Michael Larkin[1], Hui Qiang Lin[2] and John Bartlett[1]; [1]Materials and Engineering Science, Australian Nuclear Science and Technology Organisation, Menai, New South Wales, Australia; [2]Radiopharmaceuticals, Australian Nuclear Science and Technology Organisation, Menai, New South Wales, Australia.

Silica nano-particles possess several intrinsic advantages as drug carriers. They are biologically inert, have a hydrophilic surface, which reduces their detection by the reticuloendothelial System and exhibit a prolonged shelf-life in the dry state. We have developed a novel approach to synthesize silica nano-particles for the encapsulation and release of bioactive molecules. The bioactive species are incorporated, at ambient temperature, into the evolving inorganic particles using an emulsion gelation process. The particle diameter is determined by the size of the reverse micelles, which is controlled by the emulsion chemistry. The release rate of the encapsulated species is controlled by the internal structure of the spheres, which can be precisely tailored by varying the sol-gel chemical parameters. The ability to independently control the release rate and particle size renders this technology particularly attractive for passive, in-vivo targeting of different organs and tumours. This process has been used to produce mono-disperse nano-particles containing doxorubicin with controlled sizes ranging from 25-250 nm. The subsequent release of the encapsulated doxorubicin in aqueous solution exhibited zero order release kinetics for more than two weeks. Preliminary in-vivo bio-distribution results, as well as comparisons with alternative controlled-release carriers based on polymers and liposomes, will be presented.

2:15 PM I2.4
Targeted Dextran Conjugates for Delivery of Doxorubicin to Human Hepatocytes. Noreen Tasneem Zaman, Todd C Zion and Jackie Y Ying; Chemical Engineering, MIT, Cambridge, Massachusetts.

Conventional chemotherapeutic agents used in cancer treatment must be administered at suboptimal doses to avoid serious side effects, some of which can be alleviated by targeted delivery. Protein targeting, specifically, lectin (carbohydrate receptor) mediated targeting holds potential due to high specificity and affinity, rapid internalization by receptor-mediated endocytosis and relative ease of processing. Recently, nanoparticles have been used in drug delivery to increase circulation time and decrease toxicity of drugs. They remain undetected by the immune system and are internalized at a greater rate than larger particles. We have developed a novel reverse microemulsion synthesis scheme utilizing biocompatible oils and surfactants to synthesize polymeric nanoparticles for drug delivery. The purpose of this project is to synthesize targeted, stimuli-responsive nanoparticles encapsulating doxorubicin for cancer chemotherapy. We have investigated the interaction between cells and inert nanoparticles of varying sizes, expressing different carbohydrate moieties. We have found that human hepatocytes (Hep G2), which express a high surface density of galactose binding sites (asialoglycoprotein receptor), take up two to seven times as many galactose-expressing (targeted) nanoparticles as non-targeted nanoparticles. Hep G2 cells also internalize at least twice the number of the targeted nanoparticles compared to the control cell line. The uptake in both cell lines increases significantly with a decrease in particle size. We also found the relative uptake of targeted to non-targeted particles to be time-dependent in the Hep G2 cell line, but not in the control cell line. We are functionalizing dextran with galactose to verify if internalization by Hep G2 increases with the concentration of galactose residues on the polymer as well as with the spacing between the polymer backbone and residue. Doxorubicin will also be conjugated to these galactose-expressing polymers to determine if Hep G2 cells are killed more efficiently than the control cells.

2:30 PM I2.5
Glucose-Responsive Nanoparticles For Controlled Insulin Delivery. Todd Charles Zion and Jackie Y. Ying; MIT, Cambridge, Massachusetts.

Polymer nanoparticles have shown outstanding promise in facilitating the administration of therapeutic proteins such as insulin. Nanoparticles can encapsulate and release insulin over extended periods of time to reduce dosage frequency, protect insulin from harsh

conditions in the digestive tract and absorb through the intestinal epithelium to facilitate oral delivery, and deposit preferentially in the lung alveoli to enhance pulmonary delivery. Despite the attractive features of nanoparticles in improving insulin delivery, the usual mechanism of sustained release is independent of physiological blood sugar concentration. Our laboratory has recently developed a reverse microemulsion (RM) synthesis of ultrafine inorganic particles for catalytic applications and sought to apply those techniques to the synthesis of polymer nanoparticles for controlled drug delivery. This project focuses on the design and characterization of glucose-sensitive nanoparticles for stimuli-responsive insulin delivery using materials constructed from the physical interaction between a glucose-binding protein and dextran. In order to assess RM phase behavior in the presence of potentially surface-active macromolecules such as polymers and proteins, we have developed and applied a rapid screening approach to quantify the optical clarity of a large number of ternary compositions at select temperatures. The technique has allowed us to determine an optimal RM formulation for the synthesis of physically crosslinked nanoparticles as confirmed by dynamic light scattering and scanning electron microscopy. The particle crosslinking efficiency and glucose-sensitive dissolution were quantified using fluorescence spectroscopy and found to depend on the ratio of Con A to dextran, dextran molecular weight, and covalent modification of dextran with higher affinity sugars. The particle glucose-sensitivity may be adjusted from 50 mg/dl to 500 mg/dl by varying the Con A/dextran ratio and sugar binding affinity. In addition, insulin was encapsulated in select formulations and found to release up to an order of magnitude more rapidly at elevated glucose concentrations. Further experiments reveal that both nanoparticle degradation and insulin release are reversible under dynamic glucose cycling conditions. Finally, select formulations have been administered in vivo to diabetic rodent models to determine their ability to control blood sugar over extended periods of time and respond to acute blood glucose challenges.

2:45 PM I2.6
Engineered Chitosan Nanoparticles for Removal of Overdosed Toxic Drugs. Dong-Won Lee and Ronald Baney; Materials Sci. & Eng., Univ. of Florida, Gainesville, Florida.

Drug toxicity, intentional or accidental, is one of major health concerns worldwide. However, specific pharmaceutical antidotes have not been developed for majority of the drug intoxication such as amitriptyline. Amitriptyline, the most widely prescribed antidepressant and leading material for committing suicide in the U.S., was chosen as a model for overdosed drugs. Successful detoxification requires the selective and rapid adsorption of the overdosed toxic drugs from the bloodstream by systems such as bio-nanoparticle systems. The drug removal by bio-nanoparticulates is a concept in contrast with drug delivery or controlled drug release. The objectives of this work are the development of intravenously injectable chitosan nanoparticles for selective and rapid drug removal from the bloodstream and evaluation of drug adsorption efficacy. Chitosan polymers, which are naturally occurring biocompatible aminopolysaccharide, were modified with π complex forming groups, benzenesulfonyl and dinitrophenyl groups, for interaction with the aromatic groups of amitriptyline. ^{1}H-NMR was employed not only to confirm the modified chemical structure and monitor the π-π complexation by aromatic-aromatic interaction. ^{1}H-NMR study shows aromatic protons on benzenesulfonyl and dinitrophenyl groups were shift upfield with amitriptyline, suggesting the formation of π complex by aromatic-aromatic interaction between substituted groups and aromatic region on amitriptyline. HPLC data reveal that engineered chitosan systems significantly reduce the free concentration of amitriptyline from the aqueous solution. Hemolysis and thromboelastography showed the blood compatibility of the chitosan particles.

SESSION I3: Targeting and Modelling Systems
Chair: Dan Luo
Monday Afternoon, December 1, 2003
Republic A (Sheraton)

3:30 PM I3.1
Targeted Multivesicular Liposomes for Delivery of the Alpha-Emitting Isotope Generator, Actinium-225, and Its Radioactive Daughters. Stavroula Sofou[1], James L Thomas[2], Michael McDevitt[1], David A Scheinberg[1] and George Sgouros[3]; [1]Program in Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Department of Physics and Astronomy, University of New Mexico, Albuquerque, New Mexico; [3]Department of Radiology, Division of Nuclear Medicine, Johns Hopkins Medicine, Baltimore, Michigan.

Targeted alpha-particle emitters hold great promise as therapeutic agents for disseminated cancers. Their high energy deposition per distance traveled can result in cell death with as few as 1 to 3 traversals through the nucleus. In addition, the 50 to 100 micron range of alphas is consistent with the dimensions of disseminated disease, allowing for localized irradiation of target cells with minimal normal cell irradiation. One of the most promising such radionuclides, is the atomic generator actinium-225; it has a 10-day half-life, and a decay scheme that yields Fr-221 (4.9min), At-217 (32.3ms) and Bi-213 (45.6min) leading to the emission of a total of 4 alpha particles. If these daughters are confined to target cells, efficacy is likely to be increased; distribution throughout the body, is likely to increase toxicity. Antibodies have been used for targeted delivery of Ac-225. This approach, however, delivers only the parent since following alpha-particle decay all subsequent daughters are no longer associated with the antibody due to the recoil of the daughters and lack of chelation upon emission of the alphas. To overcome this problem, we have investigated the feasibility of retaining the charged daughter intermediates within engineered liposomes. Actinium-225 was passively entrapped in 100, 400 and 650nm-diameter liposomes; retention was stable, above 88% for 30 days. Daughter retention was evaluated by measuring the retention of the last daughter in the decay series, since escape of prior intermediates would also lead to Bi-213 loss. Daughter retention was liposome-size dependent. Because the daughters recoil about 100nm, retention was neither expected nor observed for the smallest liposomes. However retention was lower than expected for the larger liposomes. This was shown to be, in part, due to partition of Ac-225 to the phospholipid membrane. Actinium-225 localization to the liposomal membrane (surface) increases daughter loss due to nuclear recoil, compared to daughter loss from uniformly distributed Ac-225 atoms within the liposomal aqueous phase. To overcome this, large liposomes with entrapped smaller vesicles, containing Ac-225, were prepared. This strategy provides confinement of entrapped Ac-225 within the region of the liposomal aqueous part, away from the outer liposomal membrane, and also results in decreased radionuclide partition to the external membrane (surface), due to increased internal membrane. Multivesicular liposomes (MUVEL) were prepared and characterized for content leakage and size, over time, and entrapment efficiency. Retention of Bi-213 by MUVEL was significantly improved. We anticipate that immunolabeled MUVELs will make it possible to retain the high potency of Ac-225 while reducing the toxicity associated with untargeted daughter emissions thereby enhancing its potential for cancer therapy. This approach is ideally suited for locoregional therapy (e.g. intraperitoneal, intrahepatic artery, intrathecal).

3:45 PM I3.2
New Insights into Skin Permeation Enhancement by Fatty Acids. Mei Yin Wang[2,1], Yi Yan Yang[2] and Paul Wan Sia Heng[1]; [1]National University of Singapore, Singapore, Singapore; [2]Institute of Bioengineering and Nanotechnology, 1 Science Park Road, #01-01/10, The Aries,, Singapore.

Transdermal drug delivery is an attractive alternative to conventional oral route of drug administration. Fatty acids are common permeation enhancers used to overcome skin barrier, mainly stratum corneum (SC). The enhancement mechanism for fatty acids has been widely explored. In this study, new insights into these mechanisms have been discovered. Two solvents (propylene glycol, PG, and mineral oil, MO) of opposing nature were used as carriers for the fatty acids. Formulation uptake, FTIR, partition coefficient between isopropyl myristate and formulation, 1H NMR and skin permeation of physostigmine as a model drug were studied to evaluate possible interactions among skin, solvent used, fatty acid and drug. Contrary to what other studies have reported [1-3], it was shown here that short chain fatty acids were able to disrupt lipid bilayers when carried in MO, and affected drug permeation across the skin. The ability of the fatty acids to enter SC depended on the overall lipophilicity of formulation. It was vital that fatty acids were able to enter the SC so as to enhance drug permeation. Another important finding was that long chain fatty acids in PG provided greater permeation capacity than in MO. The cause was attributed to the ability of PG to enter corneocytes that form the bulk of SC. The low formulation uptake by SC and the ease in entering the lipid domains suggested that drug permeation for MO-based formulations could occur predominantly by the intercellular route within the SC. As for PG-based formulations, the disruption of the lipid bilayers was vital to permeation of the drug complex. Along with high formulation uptake by corneocytes and low partitioning into lipid domains of SC, it was postulated that drug permeation for PG-based formulations was via the transcellular pathway. These findings would make an important contribution to design of transdermal drug delivery systems. References 1. B.J. Aungst, J.A. Blake and M.A. Hussain. Pharm. Res. 7:712- 718 (1990) 2. B.J. Aungst, N.J. Rogers and E. Shefter. Int. J. Pharm. 33:225-234 (1986) 3. H. Tanojo and H.E. Junginger. J Disper Sci Technol. 20: 127-138 (1999) 4. M.Y. Wang, Y.Y. Yang and P.W.S. Heng. Role of solvent in interactions between fatty acids and lipids in porcine stratum corneum (submitted) 5. M.Y. Wang, Y.Y. Yang and P.W.S.

Heng. New insights into fatty acids permeation enhancement with use of different solvents (submitted)

4:00 PM I3.3
Development of a Smart Bandage: Applying Electrical Potential to Selectively Release Wound Healing Growth Factors From Cell Free Extracellular Matrices.
Susan J. Braunhut, Ekaterina Vorotnikova, Donna McIntosh, Tiean Zhou and Kenneth A. Marx; Center for Intelligent Biomaterials, Departments of Chemistry and Biological Sciences, Univ. Mass., Lowell, Massachusetts.

The extracellular matrix (ECM), composed of proteins and glycosaminoglycans, provides scaffold support and is a reservoir for signaling peptides and growth factors during wound healing. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor beta (TGF-b) are growth factors known to be synthesized and stored in the ECM by capillary endothelial cells (ECs). In this study, we investigated the controlled release of growth factors from cell-free matrices and tested the ECM-derived factors for their ability to facilitate healing of full thickness wounds in mice. ECs were grown in 8-chamber electrical cell impedance sensing (ECIS)array devices (Applied BioPhysics, Inc.) for 6 days to reach confluency and cover the gold conducting material of each chamber with ECM. The cells were removed non-proteolytically using EGTA in three successive 2-hour washes, as described (1). The cell-free ECMs were overlaid with Mg++, Ca++-free PBS and after 24 hrs the pre-electrical potential PBS (control-PBS) was collected. A second, equal volume of PBS was then added to the ECM in each well and the ECM was subjected to -0.3 V (wrt Ag/AgCl) applied electrical potential for 1 hr. The electrical potential releasate PBS (-0.3V-PBS) was collected after 23 hrs. The control and -0.3 V PBS were tested in standard proliferation assay using naive ECs as described (2). -0.3V-PBS inhibited the EC growth 48% over that of control PBS. A neutralizing antibody to TGF-b significantly blocked the action of this factor in -0.3 V PBS. Mice received two identical elliptical wounds on either flank under anesthesia and one wound was treated with control PBS and the other with an equal volume of -0.3 V PBS. Wounds were measured over 14 days using a vernier caliper. The single application of -0.3 V PBS immediately after wounding reduced the wound healing time by ∼50% compared to the matched wounds which received control PBS in parallel. These studies indicate that cell free matrices can be generated and stored as a reservoir of wound healing growth factors. Upon demand, electrical potential can be used to liberate these growth factors and apply them to wounds to facilitate wound healing. Additional studies indicate that different cell types produce matrices that vary in their growth factor content and the same matrices treated with different applied electrical potential release different types of growth factors. This work should lead to a new type of bandage that can be customized to treat different types of injury. 1. Paige, K.et.al. 1991. In Vitro Cellular & Developmental Biology, 27, 151-157. 2. Braunhut, S.J. et.al. 1992. Differentiation, 50. 141-152.

4:15 PM I3.4
Changes in the Hydrolytic Degradation of Poly(anhydride-ester) Fibers as Related to Copolymer Composition and Mechanical Properties. Kenya Whitaker[1] and Kathryn Uhrich[2]; [1]Department of Chemical & Biochemical Engineering, Rutgers University, Piscataway, New Jersey; [2]Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey.

Poly(anhydride-esters), based upon carboxyphenoxydecanoate (CPD), are biocompatible polymers which hydrolytically degrade into salicylic acid, a non-steroidal anti-inflammatory agent (NSAID). The properties of the poly(anhydride-esters) can be altered by copolymerization with *para*-carboxyphenoxyhexane (*p*CPH). Examination of three compositions (30:70, 40:60, and 50:50) is reported as a function of hydrolytic degradation. The most apparent changes are in the mechanical properties of CPD:*p*CPH copolymer fibers. The mechanical properties examined were tensile modulus at 1% strain ($E_{1\%}$), tensile strength (σ_B), ultimate elongation (ϵ_B), and toughness (E_r). For the copolymer compositions examined, the values of these mechanical properties decreased as a function of hydrolytic degradation. The 30:70 CPD:*p*CPH fibers maintained higher values for $E_{1\%}$ at all time points, as compared to the two other fiber compositions. Additionally, the 30:70 CPD:*p*CPH fibers maintained lower values for both ϵ_B and E_r relative to the two other fiber compositions. These phenomena resulted from the brittle nature of *p*CPH, the major component of the 30:70 fibers; increasing the *p*CPH concentration in the polymer lowers both tensile strength and toughness of the polymer by decreasing ductility. However, with increasing amounts of *p*CPH, the hydrolytic degradation occurred more slowly, which is reflected in the copolymers' improved ability to retain their mechanical properties. Copolymerization is therefore useful for controlling not only the mechanical properties of the polymers but, more importantly, the rate at which the polymers degrade, which determines the rate at which the NSAID is released *invivo*. This allows for control of both dosage release rate and *invivo* level of NSAID.

4:30 PM I3.5
Rapid screening of receptor-ligand interactions with all-atom Go-Model. Melik C Demirel, Engineering Science and Mechanics, The Pennsylvania State University, University Park, Pennsylvania.

Understanding the structure and function relationships for proteins and biomolecular assemblies is one of the challenges in the post-genomic era. At the molecular level, molecular dynamics (MD) simulations are very powerful. The high computational cost of MD simulations, however, is a drawback and new techniques are needed to overcome this problem. A viable alternative method to study receptor-ligand binding is the coarse-grained MD simulations of simplified models, [1-2] such as the all-atom Go-Model. [3] There have been several methodological advances and novel applications for coarse-grained calculations (i.e., Go models) on biological molecules. Go-models have given promising results for calculating the folding mechanism of real proteins. In all-atom Go-models the configurational entropy is modeled accurately, while energy terms, according to the principle of minimal frustration of proteins, can be modeled by short-range interactions between known native state pairs of atoms. We propose to use the all-atom Go-model to calculate the binding mechanism of surface proteins to their ligands which can also be used to identify drug targets rapidly. 1.Bahar, A. R. Atilgan, M. C. Demirel, et al., PHYSICAL REVIEW LETTERS 80, 2733 (1998). 2.M.C. Demirel, A. R. Atilgan, R. L. Jernigan, et al., PROTEIN SCIENCE 7, 2522 (1998). 3.C. Clementi, A. E. Garcia, and J. N. Onuchic, J MOL. BIOL. 326, 933 (2003).

4:45 PM I3.6
Diffusion of biomolecules through nanoporous inorganic membranes. Ketul C Popat, Kristen Laflamme and Tejal A Desai; Biomedical Engineering, Boston University, Boston, Massachusetts.

Nanoporous membranes are important for a variety of applications including controlled sustained drug delivery. In this study, nanoporous membranes from two different types of materials were fabricated. Silicon membranes were manufactured using microfabrication techniques whereas alumina membranes were manufactured using anodization of aluminum. Both of these membranes can be made with very well defined pore size distribution and controlled microarchitecture on size scale relevant to biological systems. These membranes have shown linear diffusion rates for three biomolecules namely; glucose, albumin and IgG over extended period of time. However, the membrane may suffer from biofouling due to non-specific protein interactions. Therefore, we have covalently coupled with poly (ethylene glycol) (PEG) to avert this limitation. We have investigated the performance of these nanoporous membranes in terms of their diffusion characteristics and protein adsorption after surface modification with PEG.

SESSION I4: Poster Session
Chair: Mark Saltzman
Monday Evening, December 1, 2003
8:00 PM
Exhibition Hall D (Hynes)

I4.1
Thermoresponsive Hydrogels on the Basis of Poly(N-Vinylcaprolactam) for Controlled Delivery of Drugs. Rinat M Iskakov[1], Erkesh Batyrbekov[1,2], Sergei Sedinkin[1], Zhibek Jumagulova[1], Esen Bekturov[1], Galym Mamytbekov[1] and Bulat Zhubanov[1]; [1]Polymer, Institute of Chemical Sciences, Almaty, Kazakhstan; [2]Insitute of Chemical Sciences, Almaty, Kazakhstan.

The aim of this work is the development of controlled delivery system immobilized by antimicrobial drug farmazin or anesthetic kazkain into a copolymer gel on the basis of N-vinylcaprolactam and sodium itaconate, that shows low critical solubilization temperature (LCST) at heating. Shinkage of the gel within a narrow range of tem-perature is provoked by increasing the hydrophobic interaction of nonpolar groups of the gel at higher temperature. That stimulates release of water from the gel surface and causes increasing of the system entropy. LCST of blank gel and one with drugs immobilized by sorbtion was studied. The blank gel shows LCST at 35C, while that of the drug immobilized gel drops up to 32,5C. The effect of temperature on release of the drug from the gel of 0.5 g into physiological solution (200 mL of buffered sa-line) at temperatures above (37C) and under (22C) LCST was studied. Halftime release of the drug above LCST (37C) was 210 min, while that under LCST (22C) detected at 32 min.

At high temperature in shrunken gel the diffusion of drug plays a limiting role. In the other hand, at low temperature the gel is swollen, so the concen-tration of the drug in the surface area is a driving force of the release. Thus, the immobilization of drugs into a thermoresponsive gel on the basis of poly(N-vinylcaprolactam) is an applicable tool to control the diffusion of drug by a little change of temperature. Such systems could be utilized for controlled delivery of bio-logically active substances in medicine and pharmacy

I4.2

Controlled Release From NIPAm Nanoparticles Containing Degradable Crosslinkers. John Vincent St. John[1], Kevin F Shannon[1], Zhibing Hu[2] and Tong Cai[2]; [1]Hydrogel, Access Pharmaceuticals, Dallas, Texas; [2]University of North Texas, Denton, Texas.

Crosslinked hydrogel nanoparticles are an effective matrix for delivery of small molecule drugs in the body. It is sometimes desirable to control the rate of release of drug molecules from a crosslinked hydrogel through a mechanism other than simple diffusion. Toward this aim, symmetrical crosslinkers have been synthesized which can be polymerized in the presence of n-isopropyl acrylamide (NIPAm) monomers to produce hydrogel nanoparticles that degrade under physiological conditions. The symmetrical, bifunctional crosslinkers are of the structure: hydroxyethyl acrylamide (HEAm)-Glycyl)2succinate, HEAm-GlycylGlycyl)2succinate, or HEAm-Lactyl)2succinate. The alpha-hydroxy acid group(s) (glycolate or lactate) within the crosslinker undergo hydrolysis at physiological pH, thereby allowing the crosslinked hydrogel to gradually erode. Variation of the electronic and / or steric nature or number of alpha-hydroxy linkages affects the hydrolysis rate and rate at which the crosslinked hydrogel degrades. pNIPAm nanoparticles (80-200 nm radius) were formed by copolymerizing NIPAm monomer with various ratios of degradable crosslinkers. Degradation was indicated with the swelling and subsequent breakdown of the nanoparticles as followed by laser light scattering. The degradation rate was a function of the type and mole percent of degradable crosslinker in the hydrogel. The particles degrade into linear fragments and the molecular weight of the degradation fragments can be varied with changes in initiator concentration and polymerization temperature. The release profiles of bromocresol green dye, and bovine serum albumin from degradable and non-degradable pNIPAm nanoparticles have been studied to determine the effect of the degradable crosslinks on drug release.

I4.3

pH-sensitivity of poly(N-isopropylacrylamide-co-Maleic Acid) for oral vaccine delivery systems. Rachael Weiss-Malik and Brent Vernon; the Harrington Department of Bioengineering, Arizona State University, Tempe, Arizona.

Oral delivery systems for proteins and other labile drugs must overcome some daunting obstacles to be efficacious. One such obstacle is the digestive environment and low pH of the stomach. Temperature-responsive, pH-sensitive polymers have been used to protect insulin in the stomach and allow release in the upper intestinal tract. Temperature/pH-sensitive polymers have been demonstrated to increase insulin release at pH 8 compared to pH 2(1). Here poly(N-isopropylacrylamide-co-maleic acid) (NIPAAm-co-MAc) is being developed for application in a new oral vaccine delivery system. It is expected that poly(NIPAAm-co-MAc) will show a difference in its lower critical solution temperature (LCST) and swelling pH sensitivity compared to poly(NIPAAm-co-acrylic acid) due to its diprotonic nature. The purpose of this study was to evaluate the effect of MAc on the LCST of poly(NIPAAm). Poly(NIPAAm-co-MAc) with 5-mol% MAc was synthesized at 60°C in THF. The LCST was approximated using solution differential scanning microcalorimetry (DSC) on 5-wt% solutions at pH's from 2 to 10. The DSC was conducted at 1°C/min from 0°C to 80°C and from 80°C to 0°C. The molecular weight was determined by GPC/light scattering. The weight average molecular weights for two polymerizations were determined to be 20,000±6600 and 25,000±8700 g/mol. The number average molecular weights for each were 15,200±6000 and 18,300±600 g/mol. DSC data is provided in the table below; a gradual increase in LCST was observed in the forward DSC scans from pH 2 to pH 5. At pH 5, the LCST reached a plateau. The backward DSC scans revealed a more observable LCST transition between pH 4 and 5, with a gradual increase between pH 2 and 4 and a plateau after pH 5. MAc is known to have two pKa values at pH's 1.9 and 6.2; the pH of the LCST transition is proposed to correspond to the deprotonation of the first -OH group. An increase in LCST at the second pKa value was absent, suggesting that chain length between charges is the major contributing factor in the LCST transition behavior of this polymer. This has been demonstrated for poly(NIPAAm-co-acrylic acid)(2). Poly(NIPAAm-co-MAc)'s diprotonic nature is expected to provide added benefits over previously used acid polymers. Poly(NIPAAm-co-MAc) has been characterized as possessing a useful LCST pH sensitivity that can be exploited for application in oral vaccine delivery systems. 1. Ramkissoon-Ganorkar, C. et al. (1999). J Control Release 59(3): 287-98. 2. Chen, G. et. al. (1995). Nature 373(6509): 49-52.

I4.4

Controlled Delivery of Analgetics from Calcium-Alginate Beads. Rinat M Iskakov[1], Erkesh Batyrbekov[1], Dmitry Boldyrev[1], Zhibek Jumagulova[1], Valentina Yu[1], Kaldybek Praliev[1], Bulat Zhubanov[1] and Keneth Berlin[2]; [1]Polymer, Institute of Chemical Sciences, Almaty, Kazakhstan; [2]Oklahoma State University, Stillwater, Oklahoma.

With the aim of the development of drug delivery systems with prolong analgetic action; the calcium-alginate gel beads as perspective implantable biomaterial have been prepared. The present study is describing the release mechanism of analgetic drugs from the alginate gel into sodium-containing medium. We prepared drug-loaded alginate gel beads on the base of various sodium-alginate with three different mannuronic/guluronic acid (M/G) ratios, those imply on the difference in time of gel disintegration. Release of the drug from the alginate gel beads in vitro in physiological solution was studied. It was shown that alginate gels of any kind of M/G ratio release the drug with S-profile type of setting free. In the first stage there is a release of drug following by the Fickian diffusion and corresponding to the square root of time. After a discontinuous time a quick release of the drug occurs during short period, which is so-called burst release. During the burst release the remaining drug has been release completely to show a flat kinetics of drug release after all. Time lag of the burst release showed strong dependence on ratio of M/G in the initial alginate matrix. The burst release of drug was initiated by disintegration of gel with following the dissolution of polymer in medium. As amount of M blocks increases, the swelling degree of gel enhances too. Thus, alginate gel beads could be a promising drug delivery device performed as an implant. The use of M/G ratio of alginate might provide a functional time control of drug release, which is important, for instance, for analgetic medicine..

I4.5

Gold clusters as potential anticancer agents. Maria Tsoli-Leis[1,3], Hubert Kuhn[3], Guenter Schmid[1] and Helmut Esche[2]; [1]Inorganic Chemistry, University of Duisburg-Essen, Essen, NRW, Germany; [2]Molecular Biology, University of Duisburg-Essen, Essen, NRW, Germany; [3]AlCove Molecular Dynamics, Gladbeck, NRW, Germany.

Gold-based compounds have been mainly used for the treatment of rheumatoid arthritis, however many studies have shown that they can be considered as potential anticancer agents with interesting IC_{50} values for a variety of cell lines[1]. The mechanism by which gold-based compounds are affecting the growth of cancer cell lines is not really understood. Some studies have shown that they can interact with DNA[2] and other studies suggest that the interaction of the these compounds with DNA is rather not strong and reversible[3]. We have investigated the interactions of a gold cluster made of 55 atoms (Au_{55}). The gold cluster is surrounded by 12 monosulphonated-triphenylphosphane molecules. *Invitro* cytotoxicity assays with a variety of human cell lines have shown that Au_{55} is much more efficient and more toxic than cisplatin an anticancer drug used clinically. In addition we have found that the presence of albumin protein can increase the IC_{50} values indicating direct interaction with albumin protein. Our molecular docking simulations further verify the direct interaction of Au_{55} with cysteine residues on albumin. In addition docking simulations have shown that gold clusters prefer to interact with the major grooves rather than the minor grooves of DNA molecules. It is not clear yet if the mechanism by which Au_{55} is toxic to the cancer cell lines is due to direct interaction with DNA, since agarose gel electrophoresis of a plasmid DNA incubated previously with Au_{55} has not shown any effects the electrophoretic mobility of DNA.

[1] C. F. Shaw, *ChemicalReviews*, 1999, **99**:2589.
[2]P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli, G. P. Speroni, *Journal of Inorganic Biochemistry, 1997,* **66***:103.*
[3]*L. Messori, P. Orioli, C. Tempi, G. Marcon,Biochemical & Biophysical Research Communications, 2001,* **281***:352.*

I4.6

Manipulation of Biomolecules by Au/γ-Fe_2O_3 Composite Nanoparticles. Takuya Kinoshita[1], Satoshi Seino[2], Yohei Otome[1], Takashi Nakagawa[1], Kenji Okitsu[3], Yoshiteru Mizukoshi[4], Tadachika Nakayama[2], Tohru Sekino[2], Koichi Niihara[2] and Takao A Yamamoto[1]; [1]Graduate School of Engineering, Osaka University, Suita, Osaka, Japan; [2]Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka, Japan; [3]Graduate School of Engineering, Osaka Prefecture University, Sakai, Osaka, Japan; [4]Department of Industrial Chemistry, Osaka Prefectural College of Technology, Neyagawa, Osaka, Japan.

We measured amount of glutathione (GSH), which was adsorbed onto

the gold/γ-Fe_2O_3 composite nanoparticles and separated by the magnetic separation technique in an aqueous phase. The composite nanoparticle consists of gold nanograins of about 5-nm diameter supported on a γ-Fe_2O_3 particles about of 20-nm, which was obtained by gamma-ray irradiation of an aqueous solution containing the γ-Fe_2O_3 nanoparticles and Au^{3+}. The mass ratio of gold and γ-Fe_2O_3 was 1:10. The GSH, a polypeptide with mercapto and amino group, was added to an aqueous suspensions of the composite nanoparticles, so that 1.1 g-particle/l and 100 μmol-GSH/l. After stirring, the suspension was separated into magnetic and nonmagnetic components by the magnetic separation technique. The amount of GSH remaining in the nonmagnetic solution was measured by the enzymatic recycling technique in order to calculate the amount of GSH adsorbed onto the composite nanoparticles. For comparison, we also measured amounts of GSH adsorbed onto monolithic gold nanoparticles and monolithic γ-Fe_2O_3 nanoparticles, both of which are of similar size and amounts in the composite sample. Thus measured amounts of GSH onto the nanoparticle samples showed that the composite nanoparticles adsorbed GSH best, which was more than the sum of those by the monolithic gold and γ-Fe_2O_3 suspensions. The typical amount absorbed was 72 μmol-GSH/l. The results indicate that the affinity between gold and GSH is not spoiled at all even when the gold particles are supported on the γ-Fe_2O_3. The GSH is considered to be connected to gold via mercapto and/or amino group and manipulated by a magnetic force exerted on the γ-Fe_2O_3 particle. The present our results are very promising from the viewpoint of application to the drug delivery system, since gold firmly combines with biomolecules such as DNA, protein and medicine via a covalent bond between gold and mercapto or amino group.

I4.7
Apatite-Polymer Composites for Controlled Delivery of BSA and BMP-2. Tseh-Hwan Yong[1], Elizabeth A. Hager[1] and Jackie Y. Ying[2]; [1]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Bone morphogenetic proteins (BMPs) are potent inducers of bone formation and provide a promising alternative to bone graft. Successful orthopedic repair by these proteins requires suitable carriers that can retain BMPs at the defect site for a sufficient amount of time to allow the recruitment, differentiation, and proliferation of bone-forming cells. Sustained release is especially important for higher mammals which have less responsive cells and take longer to heal. A carrier with low initial burst and zero-order release is also desirable to conserve and meter out the therapeutic protein at the required rate. We have prepared protein-loaded microparticles from nanocrystalline apatite (hydroxyapatite or carbonated apatite) and poly(lactic-co-glycolic acid) (PLGA) using a solid-in-oil-in-water suspension process. Processing and material parameters, such as solution viscosity, homogenization time and speed, and polymer molecular weight, were adjusted to obtain particles ranging from 20 to 70 μm. These microparticles showed tunable release of a model protein, bovine serum albumin (BSA), as well as bone morphogenetic protein-2 (BMP-2). In contrast to polymer microparticles fabricated by conventional water-in-oil-in-water (W/O/W) emulsion process, the rate and length of protein release from these composite particles can be readily modified by varying the apatite loading, polymer composition, and polymer molecular weight. Sustained release has been achieved for as short as 1 week to more than 3 months. Porous, bulk composite scaffolds have also been prepared, and exhibited an extended period of release upward of 6 months due to slower water penetration into the scaffolds. The effect on mouse pluripotent fibroblastic C3H10T1/2 cells of sustained BMP-2 release from these composite microparticles, versus burst release from polymer microparticles prepared by the W/O/W process, will also be discussed.

I4.8
Controlled release of β-estradiol from biodegradable polymer microspheres. Richard Jones, Prabha Achalla, Yohannes T Tesema and Dharmaraj Raghavan; Chemistry, Howard University, Washington, DC, District of Columbia.

β-estradiol, a naturally occurring hormone, is currently used in the treatment of postmenopausal osteoporosis, and has potential to be used in the prevention of various neurodegenerative conditions. Presently, the prescribed methods for release of β-estradiol include oral and transdermal delivery systems. Drugs encapsulated in microspheres are preferable to these methods because they can provide long term, controlled release regardless of variations in patients compliance and skin permeability. Therefore, the primary objective of this study is to investigate the role of microsphere composition on the controlled release of β-estradiol from encapsulated biodegradable polymer matrices. Microspheres of a biodegradable polymer, Poly (lactic acid) (PLA), containing 20 wt/wt% β-estradiol were prepared by adding an oil-in-water emulsion to a large quantity of H2O. The emulsion consisted of an aqueous phase containing 0.4 wt/vol% PVA solution with an organic phase containing of PLA and β-estradiol dissolved in a CH2Cl2/MeOH (90/10 vol%) solution. Microspheres prepared using this technique were found to be homogeneous in the distribution of β-estradiol in the polymer matrix. When placed in a MeOH/H2O (70/30 vol%) solvent, there was an initial burst of drug release, with 70% to 80% of the drug being released in the first 24 hours as followed by UV/Vis spectrophotometry. After the initial 24 hours, the drug release continued at a slower rate for a considerable duration. Through this study, we plan to demonstrate the effect of microparticle composition on the initial and extended release rates of β-estradiol from polymer matrices. Acknowledgement: NSF and Keck Foundation for financial support.

I4.9
Abstract Withdrawn

I4.10
Effect of Processing and Molecular Architecture on the Vesicle Formation of Amphiphilic Diblock Copolypeptides. Kelly D Hales[1], Lisa M Pakstis[1], Enrico Bellomo[2], Timothy J Deming[2] and Darrin Pochan[1]; [1]Materials Science and Engineering, University of Delaware, Newark, Delaware; [2]Departments of Materials Science and Chemistry, University of California at Santa Barbara, Santa Barbara, California.

Self-assembling amphiphilic block copolypeptides were investigated for potential use as drug delivery materials and/or other biomedical applications. Specifically, diblock copolypeptides of hydrophobic leucine (L) or valine (V) and hydrophilic lysine (K) with poly(ethylene glycol) side groups were investigated. These materials are capable of forming vesicles in dilute solution. The focus of this work was the basic characterization of these amphiphilic diblock copolypeptides in an effort to understand the self-assembly process and the resulting structures. The effect of the copolypeptide design on the resulting morphology was studied by examining diblock compositions with different block lengths and secondary structures. In addition, different processing techniques including direct dissolution, evaporation, dialysis, and sonication in both aqueous and ionic solutions were used to examine how processing parameters effect the self-assembly process. The morphology of these materials was characterized using laser scanning confocal microscopy (LSCM), cryogenic transmission electron microscopy (cryoTEM), and dynamic light scattering (DLS) techniques. It was determined that the assembly of bilayer membranes and consequent vesicle formation depends on the volume fraction of the hydrophobic block and that changing the hydrophobic block from valine to leucine resulted in a more defined structure.

I4.11
Effect of the Linker Structure on Salicylic Acid-Derived Poly(anhydride-esters). Almudena Prudencio, Robert Schmeltzer and Kathryn Uhrich; Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, New Jersey.

A series of salicylic acid-derived poly(anhydride-esters) were synthesized by melt polymerization methods, in which the components linking the salicylic acids were varied. In this work, we define the "linker" as the structure that connects the two salicylate units. As the goal was to determine the relationship between the linker structure and the physical properties of the polymer, several linkers were evaluated including linear aliphatic, aromatic and aliphatic branched structures. Polymer properties such as molecular weight, thermal decomposition temperature, glass transition temperature and water contact angle were measured. For the linear aliphatic linkers, the molecular weight increases with the chain length (or number of methylenes). The thermal decomposition temperature also increased with the alkyl chain length but the glass transition temperature decreased, likely due to the enhanced flexibility of the polymer. Polymers with the most hindered linkers were the most difficult to polymerize, yielding lower molecular weights. Water contact angles were measured to study the relative hydrophobicity of the polymers, which influence hydrolytic degradation rates. For drug delivery, our goals are to control the processing method (solution or melt), the kinetics of hydrolytic degradation, and the mechanism of delivery. The ability to manipulate the physical properties of the polymers is relevant for tissue engineering, as well as drug delivery applications.

I4.12
Segmented Polyurethanes For Controlled Delivery of Anesthetics. Yerkesh Batyrbekov, Rinat M. Iskakov, Dmitri Yuryevich Boldyrev, Valentina Yu, Kaldybek Praliev and Bulat Zhubanov; Polymer Department, Institute of Chemical Sciences, Almaty, N/A, Kazakhstan.

Controlled release of anesthetic drugs from segmented polyurethanes have been studied. Anesthetics such as kazkaine and lidokaine were

incorporated into polymeric matrix by solvent cast technique. The drug release characteristics of such systems and anesthetic properties of the drug loaded polymers were discussed. Segmented polyurethanes (SPU) with different content of hard and soft segments were synthesized by a two-step polymerization using polytetramethylene glycol, tolyene-2,4-diisocianate and 1,4-butanediol as chain extender. The obtained SPU films were contained homogeneously dissolved drugs. The revenant parameters of the drug contained SPU were following: average molecular weight 160,000-210,000; ultimate stress 350-500 kg/cm-2, ultimate strain 460-680%; content of hard segments 14.2 - 46.4 %. Polymeric mechanical properties were changed progressively with increasing of content hard segments.. The release behaviour of antibiotics from SPU films was examined by means of immersing the polymeric samples in a modeling biological medium at 37 degrees C with constant stirring. All the release data show the typical pattern for a matrix controlled mechanism. The total amount of drugs is released in 25-30 days and depends of polyurethane structure and contents of high segments in polymer. Anesthetic properties of polyurethane-based Drug Delivery Systems were evaluated in experiments on animals.

I4.13
A Study of Biocompatible Materials for Chronic Implantation on Neural Tissue for Electrical Stimulation and Chemical Drug Delivery. Claudine Jaboro[1,2], Alexander Lagman[1], Mona Safadi[1,2], Gregory Auner[1,2,5], Jie Li[3], Pat McAllister[3], Paul Finlayson[4] and Ratna Naik[5]; [1]Biomedical Engineering, Wayne State University, Detroit, Michigan; [2]Electrical and Computer Engineering, Wayne State University, Detroit, Michigan; [3]Neurosurgery, Wayne State University School of Medicine, Detroit, Michigan; [4]Otolaryngology, Wayne State University School of Medicine, Detroit, Michigan; [5]Physics, Wayne State University, Detroit, Michigan.

This study examines the development of chronic implants using novel material based devices for electrical stimulation and chemical drug delivery. The main focus is to test the biocompatibility of the electrical stimulating and chemical drug delivery device materials and structures. The materials examined were aluminum nitride (AlN), iridium oxide (IrO2) and platinum (Pt) grown on a sapphire substrate using plasma source molecular beam epitaxy (PSMBE). Glass and sapphire were also investigated as inert standards. The chronic implants were analyzed both pre- and post- implantation onto neural tissue for material degradation and device structure/tissue interactions down to the atomic level. Characterization techniques to explore structural and chemical changes on or within the material include x-ray diffraction (XRD), infrared (IR) spectroscopy, atomic force microscopy (AFM), and x-ray photoelectron spectroscopy (XPS). Preliminary data illustrates that the materials' biocompatibility is associated with structure and topography. The characterization methods and histological techniques employed have shown that surface topography such as microroughness and microfeatures in addition to chemical stability may have a direct effect on the compatibility of the chronic implants in brain tissue. Initial findings demonstrate that material that is nearly atomically smooth such as glass will cause minimal to no tissue damage when compared to a material that has significant microroughness as platinum.

I4.14
Biodegradable, Multireservoir Microdevice Drug Delivery. Grace Young Kim[1], Amy C.R. Grayson[1], Michael J. Cima[1] and Robert Langer[2]; [1]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Pulsatile drug delivery from a biodegradable, polymeric device has been reported [Grayson, et al., submitted]. An array of drug reservoirs was formed in a polylactic acid (PLA) substrate. Each reservoir was individually sealed with a polylactic-co-glycolic acid (PLGA) membrane. Drug release from the reservoirs is programmed by modifiying the chemistry and molecular weight of the membrane material. In vitro results suggest that when the membrane biodegrades to a threshold molecular weight, the reservoir is opened and the drug depot is released. Other drug delivery methods such as tablets, patches, and microspheres are often constrained to releasing a limited number of specific drug formulations during a short therapeutic window. This multireservoir microdevice, however, may have the versatility to address these limitations. Since the drugs are sealed in individual reservoirs, this multireservoir device has the potential to deliver simultaneously a wide variety of agents without being limited in formulation by each other. The enclosed microenvironment of the reservoir may allow drugs to remain stable in time periods up to a few years. Recent data on membrane characterization and the release profiles of various therapeutic agents will be presented.

I4.15
Dendrimer-like DNA as a Modular, Multi-drug Delivery Vector. Yougen Li and Dan Luo; Biological and Environmental Engineering, Cornell University, Ithaca, New York.

Many diseases are diverse in both phenotype and genotype; they are neither monogenic nor singular. Because of this diversity, drugs (including genes) that are designed to aim at a single target have seen limited success in treating complicated diseases such as cancers. Conceptually, it would be more effective to treat tumors with multiple genes/drugs that are directed against not a single, but numerous targets (e.g., delivery both genes and anti-genes along with antibodies and small chemicals). It would be even more effective if the dosage of genes/anti-genes could also be adjusted to subtypes of cancers according to, for example, molecular portraits of the gene expression pattern of that particular type of tumor. Currently, no drug delivery vector is capable of delivering genes, antigens and other entities (such as antibodies and chemicals) simultaneously within a single vector. This research focuses on constructing dendrimer-like DNA molecules that can be used as a single carrier for multi-drug (including peptide and gene) delivery. Towards that end, we have successfully created novel branching building blocks in the form of Y- and X-shaped DNA. Building upon these branched DNA structures, we have synthesized dendrimer-like DNA (DL-DNA); the formation was confirmed by electrophoresis, AFM and TEM. The synthesis is efficient and robust; the products are almost monodisperse even in the absence of purification due to their enzyme-catalyzed reactions. These multivalent DNA dendrimers can be either isotropic or anisotropic, providing great potential for linking many different chemical entities. For example, to overcome the cell plasma membrane entry barrier as well as to escape the endosomal-lysosomal pathway, Tat peptide, an endocytosis-independent cell-penetrating peptide, has been specifically conjugated to one arm of the DL-DNA via a heterobicrosslinker, SMCC (Succinimidyl trans-4-(N-maleimidylmethyl)cyclohexane-1-carboxylate). To overcome the nuclear targeting barrier, Nuclear Localization Signals are conjugated similarly to another arm of the DL-DNA. Drugs (genes, anti-genes, antibodies, etc.) can then be either covalently or non-covalently attach to the DL-DNA vector in a precisely controlled fashion by pre-designed specific DNA sequences. This versatile, water soluble, dendritic and biocompatible DNA-based vector may provide a new drug delivery platform that is capable of delivering a combination of different drugs, including genes, anti-genes, antibodies, and small chemicals.

I4.16
Functionalized Dendritic Diblock Copolymers for Receptor-Mediated Gene Delivery. Kris C Wood, Robert S Langer and Paula Hammond; Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The application of nucleotide-based therapeutics such as DNA in clinical medicine represents a technology that may potentially revolutionize the treatment of human disease. Successful delivery systems for DNA must be nontoxic, nonimmunogenic, and mechanistically must bind and condense DNA, target it to specific cells for endocytosis, protect it from lysosomal or cytoplasmic degradation, and target it to the cell nucleus for expression. In this study, we present the design and synthesis of a highly functional delivery system consisting of linear-dendritic diblock copolymers which addresses many of these challenges. We employ systems with (1) linear hydrophilic blocks such as poly(ethylene glycol) (PEG), which exhibit good aqueous solubility, chain mobility, and low cytotoxicity, and (2) dendritic blocks such as polyamidoamine (PAMAM), which have been shown to ionically condense DNA, bind non-specifically to the cell membrane, and protect DNA from lysosomal degradation by acting as a reservoir for endosomal buffering. Such systems form a protective delivery system by condensing and encapsulating negatively charged DNA in the micellar phase in solution. Further, shell-forming segments functionalized with ligands are designed for directed, receptor-mediated delivery of encapsulated genes. The resultant systems will provide a protective reservoir for therapeutic DNA to guard against premature environmental degradation and also target its delivery to cell populations with specific surface receptors.

SESSION I5: Mucosal and Micellar Delivery
Chair: Kathryn Uhrich
Tuesday Morning, December 2, 2003
Republic A (Sheraton)

8:45 AM *I5.1
Plantibodies as Mucosal Protectants. Kevin Whaley, Biophysics/Jenkins, Johns Hopkins University, Baltimore, Maryland.

Ninety percent (90%) of infectious diseases are mucosally transmitted resulting in a significant disease burden for the developed and developing world. Mucosal Protectants could close a critical gap in

prevention technologies, i.e. transmission prevention (most vaccines prevent disease, but do not prevent transmission of pathogens). Exposure to pathogens are often predictable, e.g. during intercourse (sexually transmitted infections, including HIV), during the winter ("flu" season), while traveling (traveler's diarrhea), while hospitalized or during outbreaks (SARS, rotavirus in daycare centers), suggesting that technology for transmission prevention when used intermittently could be effective and acceptable. Drugs developed as mucosal protectants are likely to be distinctly different from drugs that act systemically. Mucosal protectants are likely to be larger molecules that are minimally absorbed across (and not disruptive of) epithelium, and compatible with commensals. Biopharmaceuticals are likely to be prominent in this class (due to their potential for specificity) resulting in a drive to develop low cost/large capacity production systems. Convenient delivery systems (e.g inhalers, vaginal rings) should be evaluated with plant-manufactured pharmaceuticals, like plantibodies.

9:15 AM I5.2
Micromachined Cytoadhesive Poly(methyl methacrylate) Devices for Intelligent Oral Drug Delivery. Sarah Lynn Tao, Ka Wah Lee and Tejal Desai; Biomedical Engineering, Boston University, Boston, Massachusetts.

The rapid development of macromolecular biopharmaceuticals has placed increasing interest on advanced carrier systems for efficient oral drug delivery. Several issues are crucial for designing an effective delivery system. These include site-specific and customized delivery to achieve predictable absorption in therapeutic doses, improving the bioavailability of the drugs, and overcoming transmucosal transport and metabolic barriers. Advances in microfabrication technology have enabled the creation of entirely new classes of drug delivery devices which can possess a combination of structural, mechanical, chemical, and electronic features to surmount the challenges associated with conventional delivery systems. In this research, the strengths of microfabrication and micromachining are capitalized to create a completely novel, multifunctional microdevice from poly(methyl methacrylate) for potential applications in highly localized, tissue-specific oral drug delivery. This research has shown that PMMA drug delivery microdevices can be precisely manufactured using traditional microfabrication techniques. The devices are microfabricated to incorporate single or multiple drug reservoirs, serving as depots for any number of drugs and/or biomolecules of interest. Furthermore, the devices are asymmetrically surface modified to incorporate cell-specific targeting mechanisms. The PMMA is chemically modified by aminolysis to introduce surface amine groups to which biologically active molecules, such as avidin, can be covalently linked utilizing traditional carbodiimide coupling reagents. By further attachment of tomato lectin molecules, the PMMA is rendered cytoadhesive towards Caco-2 cells. In contrast to standard microparticulate oral delivery systems, such as microspheres and liposomes, the shape of these micromachined PMMA devices are specifically designed flat and thin to maximize contact area with the intestinal lining. This flat design also minimizes the side areas exposed to the constant flow of liquids through the intestine. Corresponding in vitro studies have shown that that these modified microdevices have increased Caco-2 cell recognition over control particles, as well as increased anchorage in comparison to PMMA microspheres. In addition, intestinal pH levels and the presence of mucus proteins do not create as great an impact on the cytoassociation of these modified PMMA microdevices as on the control devices. In conclusion, these findings demonstrate the potential advantages of utilizing micromachined, asymmetrically cytoadhesive PMMA devices for applications in oral drug delivery.

9:30 AM I5.3
Tailoring Micelle Drug Delivery Systems. Lise Arleth[1], Rex P. Hjelm[1], Beena Ashok[2], Carol F. Kirchhoff[2], Hayat Onyuksel[2], P. Thiyagarajan[3] and Jaby Jacob[3]; [1]Los Alamos Neutron Science Center, Los Alamos National Laboratory, Los Alamos, New Mexico; [2]College of Pharmacy, University of Illinois, Chicago, Illinois; [3]Intense Pulsed Neutron Source, Argonne National Laboratory, Argonne, Illinois.

Advanced drug delivery systems based on solubilization by self-assembling amphiphilic systems, may provide a means to carry lipophilic drugs safely to target tissues. By tailoring mi-celle size and shape, the drug can be delivered to the tissue and, because the carrier particles are made of material similar to the target cell membrane, they can serve to enhance the transport of the therapeutic agent into the cell. We describe a methodology of controlling micelle size and shape by mixing surfactants with different inherent interfacial curvature: egg yolk phosphatidylcholine (EYPC) and distearoylphosphatidyl-ethanolamine (DSPE) modified with poly (ethylene glycol) (PEG) of different molecular weight (2, 3 and 5 KDa). Neutron, x-ray and light scattering allow complete characterization of the micelle size and shape. The results show that the spheroid micelles formed with DSPE-PEG elongate to rod-like forms with added EYPC and that structures can be predicted from the inherent interfacial surfactant curvatures using a simple theory.

10:15 AM *I5.4
Nano-Micellelar Assembly of Multi-Functional Block Copolymers as Carrier for Gene and Drug Delivery. Kazunori Kataoka, Materials Science & Engineering, University of Tokyo, Tokyo, Tokyo, Japan.

Block copolymers with amphiphilic character, having a large solubility difference between hydrophilic and hydrophobic segments, are known to assemble in an aqueous milieu into polymeric micelles with a mesoscopic size range. These micelles have a fairly narrow size distribution and are featured by their unique core-shell architecture, where hydrophobic segments are segregated from the aqueous exterior to form inner core surrounded by a palisade of hydrophilic segments. Recently, progressive interest has been raised in the application of these block copolymer micelles as novel carrier systems in the field of drug targeting because of the high drug-loading capacity of the inner core as well as of the unique disposition characteristics in the body, which is mainly determined by the property of shell-forming segments. This presentation reviews the recent progress in the research on polymeric micelles for tumor targeted delivery systems accomplished mainly in our group. Focus is placed on the molecular design and physicochemical characterization of a series of novel polymeric micelles entrapping cytotoxic reagents, including doxorubicin and cisplatin, in the core. Longevity in blood circulation as well as enhanced tumor accumulation through EPR effect to achieve significant tumor regression will be addressed in the presentation. The concept of polymeric micelle stabilization through the formation of a hydrophilic palisade surrounding a water-incompatible core can be extended to include the case of macromolecular association through electrostatic interaction, leading to the novel type of macromolecular association: polyion complex micelles (PIC micelles). Focus of the presentation will also be placed on the use of PIC micelles as novel non-viral vector systems for delivering gene and related compounds. Indeed, blood circulation time of plasmid DNA was substantially prolonged by incorporating into PIC micelles, and eventually, expression of reporter gene in liver was confirmed. Furthermore, disulfide crosslinking of the micelle core allows selective release of incorporated plasmid DNA and in cellular cytoplasm, achieving high gene expression responding to reductive environment of intracellular compartment.

10:45 AM I5.5
Biological Properties of PEGylated Coiled coil Hybrid Block Copolymers. Harm-Anton Klok[1] and Guido WM Vandermeulen[2]; [1]Laboratoire des Polymeres, EPFL, Lausanne, Switzerland; [2]Max Planck Institute for Polymer Research, Mainz, Germany.

Recently, we(1) and others(2) have shown that conjugation of peptide sequences derived from protein folding motifs to synthetic polymers, such as poly(ethylene glycol) (PEG), allows unprecedented control over the self-assembly properties of the resulting hybrid block copolymers. In contrast to common amphiphilic block copolymers, which typically form polydisperse micellar or vesicular structures, these protein-inspired block copolymers self-assemble into supramolecular aggregates which are uniform in size and whose aggregation number can be precisely controlled via the primary structure of the peptide block. In this contribution we will present a new series of PEGylated coiled coils specifically designed in view of possible medical applications and present results of first biological experiments. Directed mutations of the amino acid sequence allowed precise control of the pH sensitivity of the coiled coil and provided access to PEGylated coiled coil superstructures which dissociate in acidic media resembling the intraendosomal or -lysosomal compartments. Haemolysis and cytotoxicity experiments did not reveal any haemolysis at pH 7.4 and indicated that the cytotoxicity of the block copolymers is comparable to that of known biocompatible polymers. Cellular uptake of supramolecular aggregates of the PEGylated coiled coils was studied using fluorescent labelled samples. Comparison of the native coiled coil sequence with the hybrid block copolymers indicated an increase in cellular uptake upon conjugation of PEG. In a final series of experiments, interpolyelectrolyte complex formation between PEGylated coiled coils and DNA was studied using gel electrophoresis experiments. The results of the biological experiments show that PEGylated coiled coils are an interesting class of novel block copolymers which hold promise for a variety of drug delivery applications, e.g. as novel, low molecular weight, gene transfection agents. (1) G.W.M. Vandermeulen, C. Tziatzios, H.-A. Klok, Macromolecules 2003, 36, 4107 (2) M. Pechar, P. Kopeckova, L. Joss, J. Kopecek, Macromol. Biosci. 2002, 2, 199

11:00 AM I5.6
The Self-Assembly Of Amphiphilic Polyethylenimines - Molecular Architecture Controls. Ijeoma Florence Uchegbu[1], Wei Wang[1], Xiaozhong Qu[1] and Laurence Tetley[2]; [1]Pharmaceutical Sciences, University of Strathclyde, Glasgow, United Kingdom;

[2]Electron Microscopy Unit, IBLS, University of Glasgow, Glasgow, United Kingdom.

The self assembly of amphiphilic polymers comprising a soluble polymer backbone and hydrophobic pendant groups yields a variety of colloidal structures e.g. vesicles and solid nanoparticles. In an effort to understand the nature of the molecular architecture controls on self assembly a variety of polyethylenimine amphiphiles have been synthesised from high molecular weight (Mw = 25kD) linear polyethylenimine (PEI) and both branched (Mw = 0.8kD, Mn = 0.6kD) and linear (Mn = 0.423kD) low molecular weight PEI. PEI is a useful gene transfer agent. Additionally colloids may be used to control drug bioavailability. A total of 24 amphiphilic polymers were prepared by the attachment of different levels of cetyl groups and in some cases an additional fixed amount of quaternary ammonium groups. All materials were structurally characterised using elemental analysis and NMR. Cetylation levels were recorded as the percentage of nitrogen residues modified by the attachment of cetyl groups. Self assembly was studied using methyl orange to probe for hydrophobic domains and any aggregates sized, imaged by electron microscopy and analysed by differential scanning calorimetry. With HMW cetylated PEI, a level of cetylation of between 33 and 49% led to the formation of 200nm vesicles, with solid 100 - 200nm nanoparticles formed from more hydrophobic materials. The attachment of quaternary ammonium groups to HMW linear PEI shifted the observation of vesicles and nanoparticles (100nm in diameter) to lower levels of cetylation (6%) while the addition of cholesterol shifted the observation of vesicles to yet lower levels of cetylation still (1%). The self assembly of LMW PEI resulted in micellar aggregates at low levels of cetylation for branched (< 26%) PEI and linear (< 42%) PEI and either nanoparticles (branched PEI) or bilayer vesicles/ bilayer fragments (linear PEI) at higher levels of cetylation. The addition of cholesterol converted the more hydrophobic LMW PEI micelles into bilayer vesicles.

11:15 AM I5.7
Self-Assembled Triblock Copolymer Hollow Nanospheres For Gene And Drug Delivery. Corinne Nardin[1,2], Durgadas Bolikal[1,2], Agnes Seyda[1,2], Larisa Sheihet[1,2] and Joachim Kohn[1,2]; [1]Chemistry, Rutgers University, Piscataway, New Jersey; [2]New Jersey Center for Biomaterials, Piscataway, New Jersey.

Advances in the design of efficient delivery systems will have a profound impact on future therapeutic practices. Our approach in this area has been to design hollow nanospheres (vesicles) made of a new, self-assembling ABA-type triblock copolymer, poly(ethylene glycol)-b-oligo(DTO suberate)-b-poly(ethylene glycol), abbreviated as POP, for the delivery of genetic materials and therapeutics. POP is derived from non-toxic building blocks, which have been shown to biodegrade in vitro. The choice of the middle block, oligo(DTO suberate), is based on its proven biodegradability as well as hydrophobicity and low glass transition temperature. The protein-repellant characteristic of the PEG end-blocks provides a protective coating against opsonization and uptake of the particles by the RES. Synthesis of POP results in a white powder (Mn = 10000 by GPC) and a hydrophobic to hydrophilic ratio of 2.6 by 1H NMR. Driven by its hydrophobicity, POP self- assembles in dilute aqueous solution into hollow nanospheres with narrow size distribution. At high polymer concentration lamellar liquid crystalline phases can be formed. The hollow nature of the nanospheres was investigated using static and dynamic light scattering. The measured radius of gyration of nanospheres is 51 nm, which is in good agreement with the largest spherical structures observed in freeze-fractured samples analyzed by TEM. The hydrodynamic radius of the nanospheres was found to be the same as the radius of gyration, indicating the vesicles are hollow in nature. The critical aggregation concentration (cac) was evaluated to be 0.26 μg mL^{-1}. This value is lower than the cac of any of the previously reported self-assembling block copolymer systems and gives this system high stability upon dilution. A cytotoxicity assay (toward UMR-106 cells) revealed no cytotoxicity in the concentration range of 0.025 to 0.5 mg mL^{-1}. Encapsulation efficiency was studied using fluorescence dyes with variable hydrophobicity. Evidence of successful loading and cellular uptake of loaded POP vesicles in tissue culture was obtained.

11:30 AM I5.8
Nanobiohybrids: Novel Drug and Gene Delivery Systems. Katherine M Tyner[1], Carl A Batt[2], W Mark Saltzman[3], Kathie A Berghorn[4], Mark S Roberson[4], Fei Hua[4], Robert F Gilmour[4], Scott R Schiffman[5], Samuel H Quinones[5] and Emmanuel P Giannelis[5]; [1]Chemistry and Chemical Biology, Cornell University, Ithaca, New York; [2]Food Science, Cornell University, Ithaca, New York; [3]Chemical Engineering, Yale University, New Haven, Connecticut; [4]Biomedical Sciences, Cornell University, Ithaca, New York; [5]Materials Science and Engineering, Cornell University, Ithaca, New York.

We have been focusing on novel nanobiohybrids as gene and drug delivery carriers. The new nanohybrids are synthesized by intercalating DNA, RNA, proteins, or drugs into the nanometer thick galleries of a biocompatible host. Charge neutralization of the biomolecules by intercalation allows for cellular uptake. Once inside the cell, the inorganic layers release the biomolecules either through dissolution or ion exchange within the neutral or slightly acidic pH environment of the body. By manipulating both the interior and exterior chemistry, nanobiohybrids are being designed for specific medical applications. In this talk, we will present our recent efforts on camptothecin and DNA nanohybrid delivery systems. Emphasis will be placed on synthesis, surface modification, cellular uptake, rate of release and efficacy studies.

11:45 AM I5.9
Investigating Block Copolymer Conformation in a Vesicle Bilayer. Jeremy Thomas Strauch, Koray Yurekli, Kishore Mohanty and Ramanan Krishnamoorti; Chemical Engineering, University of Houston, Houston, Texas.

Block copolymers (BCPs) of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) have often been used to stabilize phospholipid vesicles leading to a stable carrier for drug delivery and a mechanism to avoid annihilation by the body's defenses. However, the addition of BCPs has been shown to increase the in vitro release rate of enclosed molecules and indirectly suggests that the BCPs perturb the structure of the lipid vesicles. We have examined the mode of addition of BCPs (PEO-PPO-PEO) to single unilamelar phospholipid vesicles of dimyristoyl phosphatidylcholine (DMPC) (diameter ~100 nm) using small-angle neutron scattering. By contrast matching the scattering length densities of deuterated DMPC with appropriate mixtures of D2O and H2O, we directly probe the structure of the BCP and changes in the structure of the BCP with BCP concentration in these liposomal dispersions. We find that at low concentrations the BCP does indeed incorporate in the liposome bilayer with the incorporation into the liposome largely unaffected by the propensity for the BCP to micellize in water. However only a limited amount of BCP incorporates into the bilayer – with concentrations of BCP in excess of half the DMPC concentration leading to coexistance of BCP micelles and liposomes incorporated with BCP. Release of drugs from such liposomal formulations are influenced by the co-existence of micelles and liposomes.

SESSION I6: New Materials
Chair: Mark Saltzman
Tuesday Afternoon, December 2, 2003
Republic A (Sheraton)

1:30 PM I6.1
Developing Carbohydrate-Peptide Hybrid Copolymers as Carriers for Gene Delivery. Zhibin Guan, Mark Metzke, Soumen Maiti, Naphtali O'Connor and Kenneth Longmuir; Chemistry, University of California, Irvine, California.

We are developing a family of carbohydrate-peptide hybrid copolymers from natural building blocks as new biomaterials. Our design draws inspiration from natural biopolymers such as glycoproteins and proteoglycans. The carbohydrate-peptide hybrid copolymers were prepared through condensation polymerizations. By choosing different carbohydrate and peptide units, the hybrid polymers can be tuned to meet various biomedical applications. For one specific application, cationic carbohydrate-peptide hybrid polymers were synthesized as new polymer vectors for gene delivery. Gel electrophoresis shows that these polymers bind efficiently to plasmid DNAs and neutralize their charges. Atomic force microscopy shows that the hybrid polymers compact plasmid DNA into nanoparticles. Preliminary in vitro gene transfection to HeLa cells show that the hybrid polymer carriers can transfect genes into cells. The hybrid polymers show no cytotoxicity and will eventually degrade into their natural building blocks, i.e., carbohydrates and amino acids. We envision these carbohydrate-peptide hybrid copolymers as new biocompatible, biodegradable and functionalizable biomaterials for drug and gene delivery applications.

1:45 PM I6.2
Synthesis and Cytotoxicity of Salicylate-based Polyanhydrides. Robert Schmeltzer, Kristine Schmalenberg and Kathryn Uhrich; Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, New Jersey.

Previously, our laboratory reported the synthesis of poly(anhydride-esters) comprised of salicylic acid that were identified as a novel, efficient class of drug delivery polymers. By chemically incorporating salicylic acid into the polymer backbone via ester and anhydride bonds, polymer degradation and concomitant release of salicylic acid was observed in vitro and in vivo. Based on our success

with salicylic acid, other salicylate derivatives were investigated as candidates for inclusion into polymeric systems. The salicylate derivatives were chosen based on their use as NSAIDs, with applications ranging from mild pain therapy to tuberculosis treatments. The focus of this research is on the synthesis and cytotoxicity of these new polymers. Poly(anhydride-esters) from seven salicylate derivatives, including halogenated salicylates, aminosalicylates and thiosalicylic acid, were synthesized by melt-condensation polymerization. Cell proliferation studies were performed to assess the cytotoxicity of the polymeric materials. The polymers were first spin-coated onto glass coverslips, which were then incubated with L929 fibroblast cells in serum containing media. At 24, 48 and 72 hrs, cell viability and proliferation were determined by Calcien AM assay. By chemically incorporating these salicylate derivatives within a polymer backbone, more efficient and targeted delivery of these bioactive molecules is anticipated as a function of polymer composition and chemistry.

2:00 PM I6.3
The importance of being Multivalent: Novel star-like polymers for the detection and modulation of the T cell response. Tarek M. Fahmy[1], Kraig Haverstick[2] and Mark Saltzman[3]; [1]Biomedical Engineering, Yale University, new haven, Connecticut; [2]Biomedical Engineering, Cornell University, ITHACA, New York; [3]Biomedical Engineering, Yale University, New Haven, Connecticut.

Specific recognition of peptide/Major histocompatability (peptide/MHC) complex by the T cell receptor (TCR) governs the cellular immune response. Reagents directed against peptide/MHC complexes or the TCR have the potential of tracking and modulating either antigen presenting cells or autoreactive T cells known to mediate a variety of organ-specific autoimmune diseases. Thus these reagents would be invaluable tools in tracking the key event of T-cell recognition and the delivery of modulatory drugs to specific T cells. To enhance our ability to track and modulate the antigen-specific immune response, we have designed high avidity analogues of the T cell receptor (TCR) and Major Histocompatability Class I molecules (MHC Class I). Using star-like PEG-dendrimers as a molecular scaffold, either soluble TCR or soluble MHC were tethered to the reactive end-groups of the PEG arms on the scaffold to generate either (TCR-PEG-Dendrimer) or (MHC-PEG-Dendrimer). We demonstrate the value of this technology using the peptide-specific murine 2C T cell system. Flurorophore conjugated H-2Kb-PEG dendrimer binds 2C T cells specifically with an avidity 2 orders of magnitude higher than H-2Kb dimers. Doxorubicin conjugated 2C TCR-PEG- dendrimers downregulate peptide-pulsed T2 antigen presenting cells. The multifunctionality and increased avidity of these soluble constructs makes them potentially useful for probing the T cell response and delivering drugs to specific T cell populations.

2:15 PM I6.4
Three Structurally Distinct Biodendrimers for Use as Drug Delivery Vehicles. Meredith Taylor Morgan[1], Michael A Carnahan[1], Chad E Immoos[1], Stella Finkelstein[1], Anthony R Ribeiro[1] and Mark W Grinstaff[2]; [1]Chemistry, Duke University, Durham, North Carolina; [2]Biomedical Engineering and Chemistry, Boston University, Boston, Massachusetts.

We are interested in dendrimers synthesized from branching units that are either known to be biocompatible or natural metabolites and are thus termed "biodendrimers." Specifically, the biodendrimers used in these studies were composed of glycerol, succinic acid, adipic acid, and poly(ethylene glycol). The physical characteristics of these macromolecules, including hydrolyzable ester bonds and a large number of functionalizable end groups, suggest that biodendrimers are suitable drug delivery vehicles. 10-Hydroxycamptothecin (10HCPT), a poorly water soluble anitcancer drug (6 μM), was encapsulated within three structurally distinct biodendrimers: a dendrimer composed of glycerol and succinic acid; a layer-block dendrimer composed of glycerol, succinic acid, and adipic acid; and an A-B-A linear hybrid-dendritic copolymer composed of glycerol, succinic acid, and poly(ethylene glycol). These encapsulated complexes were fully characterized by 1D and 2D NMR experiments and quasi-elastic light scattering (QELS). A standard NCI cytotoxicity assay was used to demonstrate the efficacy of these encapsulated drug/dendrimer complexes in dramatically reducing cell viability when incubated with both human breast cancer and colon cancer cells.

2:30 PM I6.5
Multifunctional nanorods for gene delivery.
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The goal of gene therapy is to introduce foreign genes into somatic cells to supplement the defective genes or provide additional biological functions. Much of the poor transfection efficiency of non-viral vectors stems from the difficulty of controlling their properties at the nanoscale. Here we report on a novel non-viral delivery system based on nanorods that can simultaneously bind DNA plasmids and target cell receptors for enhanced internalization. The potential of this versatile gene delivery system with precise composition and size control is demonstrated in transfection studies *invitro* and *invivo*. The nanorods 100 nm in diameter and equal length of 100 nm in gold and nickel were synthesized by electrodeposition into an Al_2O_3 template. Using linkages that bind selectively to either gold or nickel, we attached DNA and a cell-targeting protein, transferrin, to the different segments. The nickel segment was surface-engineered with amine groups by reacting with 3-[2-aminoethyl)dithio] propionic acid (AEDP). Plasmids were complexed with the bound AEDP at pH 5.7. Iminothiolane was used to introduce sulfhydryl residues to transferrin, which subsequently bound selectively to the gold component of the nanorods. To further compact the bound DNA, the nanorods were incubated in $CaCl_2$. Transfection studies with HEK293 cells showed that nanorods with transferrin produced 22 % of GFP-positive cells, 2 times higher than those without transferrin; the enhancement was 3.4 times for luciferase expression level. Preliminary in vivo efficacy studies were carried out by intradermal (particle bombardment) and intramuscular (injection) delivery of the multi-component nanorods. By day 1, luciferase activity in the skin by nanorod transfection was 830-fold higher than background. This luciferase activity quickly decreased by day 4 to approximately 80-fold. Such transient expression may be suitable for genetic vaccination. In contrast, intramuscular delivery of the nanorods by injection instead of gene gun bombardment resulted in only 17-fold higher expression than the control muscle by day 1. However, this luciferase activity was more prolonged and the transgene expression rose to 85-fold higher than background by day 21. Using molecules with end-groups that selectively bind to different metals, specific functionalities can be introduced to individual segments in the nanorod. Here we have used differential binding to attach plasmids and a cell-targeting protein to spatially separated regions of the delivery system. These versatile nanorods offer an interesting alternative to current inorganic non-viral vectors in gene therapy. Acknowledgements This work was supported by DARPA/AFOSR (under grant number F49620-02-1-0307)

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Nanoporous Alumina as drug delivery material.
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Local drug delivery is the dominated by polymeric materials either being biodegradable or not. The major advantage of polymers is their diversity and the ability to easily manufacture chemical modifications to adjust the properties to different applications in the field of local drug delivery. On the other hand when trying to change drug and/or delivery time very often a complete new polymeric materials has to be designed to meet the desired specifications. Beside those organic materials in medical device technology inorganic matter is commonly used for example in cardiology (stents), oncology (seeds), for dental implants, and orthopaedics (joints, nails and others). Many of those materials have been proven to be biocompatible like for example stainless steel, titanium or aluminium oxide. Combining the need for materials suitable for drug delivery and the good biocompatible of inorganic matter brought us to the idea of using nanoporous alumina as a drug delivery material. This material is made by electrochemical oxidation of Aluminium. During this process pores of desired geometry (diameter between 15 nm and 500 nm and lengths up to 300 μm) can be made and if necessary chemical being modified using silane chemistry. The unique pore structure allows control of delivery rates of drugs just by varying aspect ratios of the pores. This technology has already been proven to be safely applied to stents and has been implanted into 30 patients. The technology is furthermore used to bind radionuclides like 125Iodine in the pores. Those implants are used to treat early prostate cancer by brachytherapy avoiding major severe side effects. We will report on the formation of the materials, the biocompatibility and the clinical application so far.