SYMPOSIUM NN

Biomaterials for Drug Delivery

November 27 – 29, 2000

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SESSION NN1: STIMULI-SENSITIVE POLYMERS AND PHASE BEHAVIOR RELATED TO DRUG DELIVERY
Chair: Surya Mallapragada
Monday Morning, November 27, 2000
Republic A (Sheraton)

8:30 AM *NN1.1
TWO PHASE POLYETHYLENE GLYCOL-POLYSILOXANE NETWORKS FOR DRUG DELIVERY - WHAT WE LEARNED WITH TRICYCLIC ANTIDEPRESSANTS. Edward W. Merrill, MIT, Dept. of Chem. Engr., Cambridge, MA; Cynthia Sung, Human Genome Sciences, Bethesda, MD; Edward Ellis, Vista Scientific, Inc, Andover, MA.

A copolymer of dimethyl siloxane and glycidoxypropylmethylsiloxane having about six epoxy groups per molecule, hereafter designated PSX,was used to end link polyethyl ene glycol of 2000, 8000, or 20000 Dalton mol. wt. under cationic initiation. The PSX also polymerixed with itself forming a cross-linked hydrophobic phase co-continuous with the hydrophilic PEG phase. The mass ratio of PEG to PSX was varied from 1/4 to 2. Equilibrium water content varied from 20 to 80 percent by weight. Two single phase networks were made as extreme limits: pure PSX via cationic initiation and pure PEG by radiation cross-linking. The uptake and release of five tricylic antidepressants were determined as functions of network composition and of the relative hydrophobicity of each TCA. Principal findings were: (1)the two phase PEG-PSX networks take up more TCA than either limiting single phase network; (2)the more hydrophbic the TCA, the higher is its partition into a PEG-PSX network;(3) the higher the PSX fraction in a network, the greater was the retention of the TCA. Because of these uptake/release characteristics, the PEG-PSX network is being evaluated as a conjunctival insert to release ophthalmic drugs, such as the polycyclics ofloxacin and prednisolone. With an equilibrium water content of about 50%, a PEG-PSX network readily conforms to the eye.

9:00 AM *NN1.2
NEW COMPLEXATION-SENSITIVE HYDROGELS FOR ORAL PROTEIN DELIVERY. Nicholas A. Peppas, Madeline Torres-Lugo, Aaron C. Foss, Daphne Robinson, Cristina Donini, Marcos Garcia, Purdue University, School of Chemical Engineering, West Lafayette, IN.

New complexation copolymer networks of poly(methacrylic acid) grafted with poly(ethylene glycol) have been shown to be excellent carriers for protein delivery due to their pH-sensitive swelling behavior as a result of the formation of reversible interpolymer complexes stabilized by hydrogen bonding between the carboxylic acid protons and the etheric groups on the grafted chains. Additionally, the presence of the PEG grafts serves as a stabilizer for entrapped peptides and proteins. Because of the complexation phenomena in these networks, the characteristic pore size in these gels is an order of magnitude greater in the uncomplexed state than in the complexed state. Because of their oscillatory swelling behavior, these gels have been used as oral carriers for insulin and calcitonin where the release of the bioactive agent in the intestine is preferable. Upon oral administration of insulin loaded gels, the blood glucose levels in rats were significantly reduced due release of insulin in the upper small intestine. Most recently, the cytocompatibility of the P(MAA-g-EG) hydrogels was evaluated in Caco-2 cells which have been used as an intestinal epithelial cell model for drug absorption studies. The powdered hydrogels exhibited no significant cytotoxic effect at the dose of 10 mg/ml. On the other hand, the transepithelial electrical resistance of the Caco-2 cell monolayers showed a reversible drop during exposure to the hydrogels at the same dose, suggesting that these hydrogels may bring about an opening of the tight junctional paracellular pathway which is a major permeation route for the protein drugs.

9:30 AM *NN1.3
DESIGN OF pH-SENSITIVE POLYMERS TO ENHANCE INTRACELLULAR TRAFFICKING OF PEPTIDES, PROTEINS, ODN'S AND DNA. Allan Hoffman, Niren Murthy, Chuck Cheung, Chantal Lackey, Pat Stayton, University of Washington, Bioengineering Department, Seattle, WA; Oliver Press, Nelson Fausto, Jean Campbell, University of Washington, School of Medicine, Seattle, WA.

The intracellular trafficking of drugs is a critical barrier to the efficacy of drugs that are degraded by lysosomal enzymes. Such drugs include peptides, proteins, oligonucleotides and DNA. The design and synthesis of carriers which can enhance the transport of endocytosed drugs from the endosomal compartments into the cytoplasm is thus an important goal. The pH of an endosome is lower than that of the cytosol by 1-2 pH units, depending on the stage of endosomal development. This pH gradient is a key factor in the design of membrane-disruptive polymers which could enhance the endosomal release of drugs. Such polymers should disrupt lipid bilayer membranes at pH 6.5 and below, but should be non-disruptive at pH 7.4. In addition, they should be capable of carrying targeting moieties to localize the therapeutics to specific cells. We will describe two different approaches to the design of pH-sensitive polymers and then present the new polymeric compositions that we have developed to deliver gene and protein therapeutics.

10:30 AM NN1.4
SYNTHESIS AND CHARACTERIZATION OF ENVIRONMENTALLY RESPONSIVE CORE-SHELL HYDROGEL NANOPARTICLES. Clinton D. Jones, Christina Baker, L. Andrew Lyon, Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, GA.

We report the synthesis of environmentally responsive hydrogels as nano-sized (30-300 nm) particles with core-shell morphologies. Composed of co-polymers of N-isopropylacrylamide with various co-monomers, these materials can be designed to render the core and shell responsive to different stimuli or to different magnitudes of the same stimulus. The measured phase transitions reflect the degree to which the two materials interact and thereby modulate the responsivity of the particle as a whole. Characterization of these materials is accomplished via dynamic light scattering, differential scanning calorimetry, electron microscopy, and fluorescence spectroscopy. Prospects for the use of these particles in the assembly of new biomaterials is discussed.

10:45 AM NN1.5
PHOTOINDUCED RELEASE OF STERICALLY STABILIZED LIPOSOMES' CONTENTS. Anja Mueller, Bruce B. Bondurant, Paul A. Spratt, David F. O'Brien, University of Arizona, Dept. of Chemistry, Tucson, AZ.

Liposomes are useful for delivery and buffering of drugs in the body. Liposomes that are sterically stabilized with poly(ethylene glycol) (PEG-liposomes) have an increased circulation time and are therefore more effective in delivering therapeutic agents to the interstitium of the tumor site. During circulation PEG-liposomes must not release the drug. After accumulation of the liposomes in the interstitium of the tumor site a trigger for release is desirable. An attractive trigger is light, because methods for the delivery of light to tumor cells have already been developed for photodynamic therapy. Therefore, the photoinduced destabilization of liposomes offers an attractive method to couple the temporal and spatial control of light to drug delivery. Bondurant and O'Brien (J. Am. Chem. Soc. 1998, 120, 13541-13542) showed that UV induced crosslinking of lipids could destabilize certain PEG-liposomes. With an appropriate choice of bilayer components the release of the PEG-liposomes' contents is efficient and fast. Visible light would be more useful, though, since it is less toxic and penetrates deeper than UV light. Here we show that photochemically induced destabilization of PEG-liposomes can be sensitized to visible light by the incorporation of a cyanine dye into the bilayer wall of PEG-liposomes. Two-photon-dyes which absorb at longer wavelengths are also being examined as sensitizers for PEG-liposome destabilization.

11:00 AM NN1.6
ELASTIN-LIKE POLYPEPTIDES AS THERMALLY TARGETED DRUG CARRIERS. Dan Meyer, Ashutosh Chilkoti, Duke Univ, Dept of Biomedical Engineering, Durham, NC; Michael Zalutsky, Duke Univ Medical Center, Dept of Radiology, Durham, NC.

Elastin-like polypeptides (ELPs) are polymers of the pentapeptide sequence Val-Pro-Gly-Xaa-Gly (where the "guest residue" Xaa can be any amino acid, in any fraction, except Pro). ELPs are an interesting class of polypeptides because they are soluble in aqueous solutions below a specific transition temperature (T_t), but, upon heating above T_t, they undergo a phase transition that results in their hydrophobic collapse and aggregation. To investigate thermal targeting of conjugated drugs to solid tumors, we synthesized a 60 kDa ELP with a T_t of 40°C, which was specified by adjusting the identity and fraction of guest residues. Because the T_t of this polypeptide is greater than normal body temperature, we hypothesize that, as a drug carrier, it will remain soluble when injected systemically. However, upon vascular transport to a solid tumor that is externally heated to $T_{tumor} > T_t$ by focused ultrasound or microwave energy, the ELP carrier will undergo its transition and accumulate through hydrophobic interactions. Here, we present results of tissue distribution studies for radiolabeled ELP carriers that were injected

into nude mice with implanted tumors (human glioma D54MG). We observed a two-fold increase in ELP accumulation versus unheated animals when the tumor-bearing limb was heated by immersion in a temperature-controlled water bath. Stringent controls show that the observed enhancement is largely caused by the phase transition of the ELP carrier rather than by nonspecific effects of hyperthermia (e.g., increased vascular permeability). Ongoing optimization studies seek to determine the effect of ELP MW, concentration, and administration protocol (e.g., bolus vs. low-level infusion) on targeting efficiency.

11:15 AM NN1.7
WATER SOLUBLE COPOLYMERS CONSISTING OF TERTIARY AMINE AND CARBOXYLIC ACID PENTANT GROUPS.
Mohammad M. Bari, Cherng-ju Kim, Temple University, School of Pharmacy, Philadelphia, PA.

This study was to synthesize new water-soluble coploymers, composing of tertiary amine and carboxylic acid pendent groups, for oral drug delivery systems. The copolymers prepared with different ratios of tertiary amine (i.e., dimethylaminoethyl methacrylate, diethylaminoethyl methacrylate) and carboxylic acid (i.e., acrylic acid, methacrylic acid) were swollen in de-ionized water and freeze-dried before obtaining fine powders. Drug release experiments with various drugs were carried out with compressed tablets in pH's of 1.5 and 7. Effects of various variations of drug carriers and drug properties on drug release kinetics were evaluated: drug solubility, drug loading, ionic types of drugs, polymer composition, types of monomers, etc. In general, zero-order release kinetics were obatained in both pH 1.5 and 7 for a long period of time. The release of drugs in pH 1.5 is not significantly different even with varying solubility and ionic status of drugs. However, different drug release profiles in pH 7 were observed with different types of drug and solubility. Molar ratios of monomer composition and types of teriary amine and carboxylic acid significantly influence drug release kinetics.

11:30 AM NN1.8
MODIFIED POLOXAMER POLYMERS FOR pH-SENSITIVE DRUG DELIVERY.
Brian C. Anderson, Surya K. Mallapragada, Iowa State University, Department of Chemical Engineering, Ames, IA; Valerie V. Sheares, Iowa State University, Department of Chemistry, Ames, IA.

Poloxamers [poly(ethylene oxide)-b-poly(propylene oxide)-b-poly (ethylene oxide)] have been investigated in recent years for drug delivery applications due to their zero order dissolution kinetics in an aqueous gel state and drug solvency capabilities. Poloxamers undergo thermoreversable gelation at moderate temperatures, making them responsive to temperature changes in their surroundings. Polymers with similar gel-forming characteristics were synthesized that also contain an ionic monomer to make the gels responsive to pH changes in addition to temperature changes. This increases the gel dissolution at lower pH levels. These polymers were characterized both for physical properties and drug delivery potential and were compared to poloxamer polymers. The influence of the relative amounts of both the poly(ethylene oxide) portion and ionic portions was also investigated to optimize the drug release rates.

11:45 AM NN1.9
TEMPERATURE-SENSITIVE POLYMER-NANOSHELL COMPOSITES FOR PHOTOTHERMALLY MODULATED DRUG DELIVERY.
Scott R. Sershen, Jennifer L. West, Rice University, Department of Bioengineering, Houston, TX; Sarah L. Westcott, Naomi J. Halas, Rice University, Department of Electrical and Computer Engineering, Houston, TX.

Composites of thermally-sensitive hydrogels and optically active nanoparticles have been developed for photothermally modulated drug delivery. Copolymers of N-isopropylacrylamide (NIPAAm) and acrylamide (AAm) exhibit a lower critical solution temperature (LCST) that is slightly above body temperature. When the temperature exceeds the LCST, the hydrogel collapses, causing a burst release of any soluble material held within the hydrogel matrix. Gold-gold sulfide nanoshells, a new class of nanoparticles designed to strongly absorb near infrared light and convert it to heat, have been incorporated into poly(NIPAAm-co-AAm) hydrogels to initiate a temperature change with light. The nanoshells consist of a thin layer of gold (~4 nm in thickness) surrounding a gold sulfide core (~40 nm in diameter), and altering the core/shell ratio allows the absorption of the nanoshells to be tuned over the visible and near IR spectrum. Light in the water window, a gap in the absorption spectrum of tissue between the chromophores (~800 nm) and water (~1200 nm), can be applied externally, pass through tissue, then be converted to heat upon interacting with the nanoshells embedded in the NIPAAm/AAm hydrogel. Significantly enhanced protein release from composite hydrogels was observed in response to irradiation at 832 nm, relative to NIPAAm-co-AAm hydrogels exposed to irradiation or composite hydrogels not exposed to irradiation. Additionally, the nanoshell composite hydrogels can release multiple bursts of protein in response to repeated near IR irradiation, allowing drug delivery to be turned on and off at will.

SESSION NN2: PROTEIN AND PEPTIDE DELIVERY
Chair: Mark A. Tracy
Monday Afternoon, November 27, 2000
Republic A (Sheraton)

1:30 PM *NN2.1
CONTROLLED RELEASE OF PROTEINS FROM EXTRUDED RODS.
Jorge Heller, John Barr, Steve Y. Ng, Hui-Rong Shen, Advanced Polymer Systems, Redwood City, CA; Alexandra Rothen-Weinhold, Robert Gurny, University of Geneva, School of Pharmacy, Geneva, SWITZERLAND.

The need to develop delivery systems for peptides and proteins where full protein activity is preserved is not well recognized. However, the development of such systems in fraught with difficulties, and dominant among these is the inability of proteins to maintain full activity when exposed to an organic solvent/water interface. Because conventional microencapsulation methods are based on the use of organic solvents and water, such methods are not generally useful for proteins that must retain their tertiary structures to preserve biological activity and specialized microencapsulation methods that avoid an aqueous phase have been developed.
Since many dry proteins have excellent thermal stability, a viable fabrication method is extrusion of intimate mixtures of micronized protein and finely powdered polymer. This method avoids exposure of the protein to organic solvents, and especially to an organic solvent/water interface. However, to be generally useful, this method needs a polymer where softening temperatures and erosion rates can be accurately adjusted to the desired values. We have described such a polymer and by proper choice of monomers, polymers that can be extruded at temperatures no higher than 70°C and where erosion rates can be adjusted to the desired values can be prepared. We have studied the in vitro release of a model protein, FITC-BSA, from 1 mm diameter rods cut to 10 mm length. Results of these studies indicate that FITS-BSA is released at linear rates with concomitant weight loss indicating that BSA release is completely erosion controlled and that the erosion process occurs by surface erosion. There was, however, a substantial induction period before BSA release took place and we will describe methods that decrease, or eliminate this induction period.

2:00 PM *NN2.2
LIPID COMPOSITE MATERIALS FOR NANOPARTICLES DELIVERY SYSTEMS.
Pierandrea Esposito, Istituto di Ricerca C. Serono - Drug Delivery System, Colleretto Giacosa, Turin, ITALY.

The use of lipids as materials for matrix type, nanoparticulate carriers manufacturing has gained considerable interest in the pharmaceutical field. They are in general GRAS materials of low or medium cost, low toxicity, and high biocompatibility. Lipids occur in a variety of compositions which influence their physico-chemical and biopharmaceutical properties. Lipid blends, prepared in controlled conditions, can further expand the potential of application of such materials to nanoparticles formulation development. Such blends, at defined compositions, can originate lipoid materials with specific surface and bulk properties. Lipid nanoparticles based on such compositions may find favourable applications in specific drug delivery applications (i.e. oral delivery of peptides). Objective sof the work are: To characterize the surface and bulk properties of the lipidic blends used for nanoparticles preparation To study how such characteristics would affect the nanoparticles properties To investigate the potential of lipid nanoparticles as a carrier for peptide drugs for the oral route Materials such as fatty acids, phospholipids, triglycerides, PEG-oylated glycerides can be used.The surface properties of thin films of lipidic blends were characterized by contact angle method: surface free energy, surface polarity or surface fractions of components were calculated, according to Gibbs or Cassie-Baxter methods (2). Phase diagrams of significant blends were constructed by DSC analysis, supported by hot stage microscopy observations. Lipid nanoparticles were prepared from the characterized blends. Particle size (measured by photon correlation spectroscopy) varied between 180 and 400 nm. The Zeta-potential values were linearly related to the surface parameters of lipidic blends, such as surface polarity or surface fraction of components. Surface microviscosity of particles was also studied by mean of polarized light spectrofluorimetry. TEM analysis evidenced the differences in nanoparticles morphology. Salmon calcitonin (sCT) was incorporated into nanoparticles prepared using lipidic blends of different properties. sCT-Nanoparticles suspensions were orally administered in vivo to Rh monkeys, at a single dose of 80 UI/Kg. Significant peptide amounts were found in plasma up to 7 hours after dosing, with corresponding reductions in total and ionized calcium concentrations. The results show that the

oral absorption of a peptide drug can be favoured by incorporating it into lipid nanoparticles. Furthermore, the modification of the surface properties of nanoparticles towards the decrease of total surface free energy, and the increase of surface polarity seems to enhance the absorption of salmon calcitonin.
References:
(1) A.T. Florence, Pharm. Res., 14, 3, (1997).
(2) T. Canal, I. Colombo, M. Lovrecich, J. Controlled Release, 27, (1993) 19-26.
Work performed at Vectorpharma International SpA, Business Innovation Center, Via del Follatoio 12, 34148 Trieste, Italy.

2:30 PM *NN2.3
BIODEGRADABLE POLYMERS, AND ENVIRONMENTALLY SENSITIVE HYDROGELS FOR THE CONTROLLED DELIVERY AND SOLUBILIZATION OF PROTEINS, VACCINES AND CONVENTIONAL DRUG MOLECULES. Gaylen Zentner, Macromed Inc., Salt Lake City, UT.

Controlled release drug delivery systems (DDS) based on biodegradable polymers in the form of environmentally sensitive gels and microspheres will be presented. Polymer technologies that effectively solubilize poorly soluble drugs are included. The PK/PD and in vitro properties of DDS for cancer (paclitaxel, hormones, cytokines), growth promotion (hGH, pGH), and vaccine (hepatitis B) therapies are the focus of this presentation. Safety/toxicity data on key polymeric components of these DDS are included.

3:30 PM *NN2.4
BIOMATERIALS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE. Michael Lysaght, Brown University, Providence, RI.

Tissue engineering appears likely to represent the next generation of substitutive medicine. It relies upon devices and therapies which utilize living cells as therapeutic reagents and combine living cells with synthetic or naturally-occurring biomaterials. Applications may involve replacement or regeneration of structural body components (eg, skin, cartilage, bone) or substitution of the function of a metabolic organ with a biohybrid equivalent (eg. pancreas, liver). Biomaterials for tissue engineering and regenerative medicine may be hydrophilic or hydrophobic, permanent or temporary, bioactive or inert. Although numerous candidates have been proposed and investigated, those applications which have reached the clinic rely upon a relatively few biomaterials ($\sim$15 in aggregate) , most of which have a successful history in earlier medical applications

4:00 PM NN2.5
SUBCUTANEOUS ADMINISTRATION OF INSULIN LOADED MICROSPHERES FOR SUSTAINED DELIVERY. Danielle Abramson, Amit Ayer, Edith Mathiowitz, Brown University, Department of Molecular Pharmacology, Physiology and Biotechnology, Providence, RI.

Current treatment for type I diabetes includes multiple subcutaneous (SQ) injections of unencapsulated insulin, which provides an initial therapeutic dose, but is short lasting. The insulin bypasses the liver, going directly into the systemic circulation where it enters peripheral tissues, exposing them to high insulin levels. A large amount of this insulin is degraded and unavailable for use. In order to mitigate some of the problems associated with unencapsulated insulin, we have developed a sustained biodegradable delivery system. Studying various types of insulin-loaded microspheres (ILMS) invitro and in-vivo, we were able to find a system that provides an initial therapeutic dose of insulin followed by a sustained stable insulin level. The most effective SQ delivery system will deliver the highest percentage of active insulin to target tissues at a rate which insulin can be utilized. Poly(fumaric-co-sebacic) (P(FA:SA)20:80) microspheres (M_n=1-5μm) were prepared by the PIN technique [1]. Microspheres were fabricated at various insulin loadings with micronized insulin or regular insulin crystals. Rats (200-300g male, CD strain) were fasted overnight and allowed free access to water. The rats were anesthetized using an isoflurane gas chamber. Blood was collected via tail bleeding. After the initial blood was collected, a SQ injection with either ILMS or unencapsulated insulin suspended in 1.0mL of saline was administered. Blood was collected for up to nine hours. Plasma samples were analyzed for both glucose and insulin levels. The P(FA:SA) microspheres loaded with 3% insulin showed a large burst effect, which corresponded to a quick drop in glucose levels followed by a slow rise to initial fasting glucose levels. Microspheres made from P(FA:SA) loaded with micronized insulin at 2% loading, exhibited no apparent sharp drop in glucose levels, but instead a slow steady release of insulin corresponding to a slow drop in glucose levels for up to nine hours. The micronization of insulin facilitates efficient encapsulation within the microspheres, thus reducing the burst effect and establishing a sustained release delivery system. We were successful in fabricating a sustained-release subcutaneous system that increased the duration of insulin action beyond that of conventional insulin injections while reducing the negative large burst effect. Future work on enteral delivery will be started shortly to establish bioavailability parameters for the same microspheres.

4:15 PM *NN2.6
USING A COMBINATORIAL LIBRARY OF DEGRADABLE POLYARYLATES TO OPTIMIZE PEPTIDE-MATRIX INTERACTIONS IN DRUG DELIVERY SYSTEMS. Debbie M. Schachter, Joshua Simon, Joachim Kohn, Rutgers University, Dept of Chemistry, Piscataway, NJ; Satish Pulapura, Arvid Viswanathan, Advanced Materials Design, LLC, Piscataway, NJ.

Delivery systems based on degradable polymeric matrices that can release high payloads of water-soluble peptides with reproducibly programmable delay times have been formulated using a combinatorially designed library of polyarylates in which the material properties of the polymers can be varied in a predictable and systematic fashion. Tyrosine-derived polyarylates are alternating copolymers of a diphenol and diacid linked by ester bonds. These polymers are readily processible, biocompatible and degrade in vivo. We had shown previously that high loadings of INTEGRILIN, a water-soluble cyclic heptapeptide (used clinically as an antithrombic drug) can be incorporated into various polyarylates and that hydrogen bonding interactions between the peptide and the polymer can prevent the release of the peptide from the matrix. Following up on this initial observation, we now show that it is possible to vary the degree of hydrogen bonding by small changes in the polymer structure. Using the available library of polyarylates, a wide range of different release profiles were obtained, including delayed release and pulsatile release profiles. Specifically, it was possible to formulate burst-free matrix systems with loadings of peptide as high as 50% (w/w) of the water-soluble INTEGRILIN. Choosing appropriate polymers from the library, highly loaded systems could be prepared that trapped the peptide within the matrix so that only a trace of peptide was released even after 77 days. In contrast, other members of the polyarylate library provided formulations that released the peptide with a controllable delay time. The length of the delay was clearly related to the molecular composition of the release matrix and ranged from no delay to a delay of over 60 days before release was observed. The precise control over the length of the lag time allows the design of a pulsatile delivery system using combinations of different polymers.

4:45 PM NN2.7
PROTEIN RELEASE FROM MULTI-LAYERED POLYMER MICROSPHERES FABRICATED VIA SOLVENT REMOVAL. J. Godbee, D. Vázquez and E. Mathiowitz, Department of Molecular Pharmacology, Physiology & Biotechnology, Brown University, Providence, RI.

Introduction: Previous research in our lab has shown multi-layered microspheres to have a lower initial burst of drug than the release obtained from single-layered microspheres of the same polymer. Solvent removal is a method that provides us with a useful way to encapsulate proteins without exposure to an aqueous environment, which reduces protein losses to the non-solvent during encapsulation. The purpose of this study is to investigate the effects of polymer solution concentration and drug loading on the release profiles from multi-layered microspheres fabricated via solvent removal. In the interest of having controlled release over several months, poly L-lactic acid (PLLA), and poly (fumaric-co-sebacic anhydride)20:80 (PFASA), were selected for these studies. These two polymers readily phase separate, which is a pre-requisite for the one-step multi-layered fabrication method. **Methods:** We used polymer solutions of varying concentrations (5%, 10%, and 15% w/v). Spray-dried FITC-labeled bovine serum albumin (BSA) was used as the model protein which was loaded into the PFASA at varying concentrations (5%, 10%, 15%, and 20% w/w). The FITC-BSA-containing solution was sonicated to create an emulsion, the two polymer solutions were then mixed into one vial, shaken vigorously and poured into the stirring non-solvent solution. Petroleum ether was added immediately to complete the removal of the organic solvent from the polymer solution droplets and harden them into microspheres. **Results:** The polymer solution concentration had a large effect on the initial burst. The 15% solutions had initial bursts at 2.4% and 5% of the theoretical value and the 10% solutions had greater than 3 times as high of a burst at 15.8% and 23.5% of the theoretical value. The drug loading did not have much of an effect on the initial burst between the 10%, 15%, and 20% loaded spheres. However, the spheres with 5% drug loading had an initial burst at least twice the value of the 10%, 15%, and 20% samples at 5 minutes. Additionally, the release profiles of the 10%, 15% and 20% samples varied: decreasing release with increasing drug loading. **Discussion:** Based on our findings we conclude that the lowest burst will be achieved from spheres fabricated from 15% solutions. The drug loading should be between 10% and 20%, depending on the desired release profile.

SESSION NN3: TISSUE AND GENE DELIVERY
Chair: Richard Korsmeyer
Tuesday Morning, November 28, 2000
Republic A (Sheraton)

8:30 AM *NN3.1
MICROENCAPSULATION OF CELLS USING SYNTHETIC POLYMERS. Michael V. Sefton, University of Toronto, Institute of Biomaterials and Biomedical Engineering, Toronto, CANADA.

Microencapsulation in a polymer membrane is a means of isolating cells from the immune system, thereby enabling the transplantation of mammalian cells without immunosuppression and the use of xenogeneic or genetically engineered cells. The cells are transplanted to correct a disease state by the delivery of a cell product, typically a protein. Insulin from pancreatic islets is an example of this mode of therapy. Unlike conventional drug delivery devices, the cells have an inexhaustible supply of the protein (pending cell viability) in an intrinsically stable form and without the problem and expense of protein purification. Furthermore, the protein is delivered at a rate determined by the normal physiology of the cells which might involve regulation by glucose level (islets) or potassium concentration (dopamine secreting cells) or cytokine levels (antitrypsin). Successful microencapsulation and cell transplantation requires (1) high cell number and viability (2) control of cell function (through the extracellular matrix, for example) and (3) maintenance of function for extended duration. These properties of encapsulated cells are illustrated with the use of hydroxyethyl methacrylate- methyl methacrylate copolymer (HEMA-MMA). This presentation will focus on the material problems associated with this type of tissue engineering.

9:00 AM NN3.2
INCREASING GENE EXPRESSION LEVEL WITH ENGINEERED PEPTIDES FOR NON-VIRAL GENE TRANSFER TO NON-DIVIDING ENDOTHELIUM. Haiching Ma, Jingya Zhu and Scott L. Diamond, Institute for Medicine and Engineering, Dept of Chemical Engineering, University of Pennsylvania, Philadelphia, PA.

The largest obstacle toward therapeutical applications of non-viral gene therapy is their poor gene delivery efficiency. This is especially true in non-dividing cells, such as the vascular endothelium. In general, there are three major steps that affect the gene delivery efficiency: endocytosis, endosome escaping and nuclear targeting, in which, the last step is often the rate-limiting step. Unlike the dividing cell, the low division rate of endothelium makes them a difficult target for nonviral gene transfer. To overcome this, we tested the nuclear importing function of non-classical nuclear localization signal peptide, M9 sequence of hnRNP A1 (38 amino acid) in confluent bovine aortic endothelial cells (BAEC). The full length M9 peptide alone has very low plasmid binding activity and can only slightly increase marker gene expression. After linking the M9 peptide with a cationic tail, the chimera peptide formed tight peptide/DNA complexes at 1:1 (w:w) ratio, which is able to protect plasmid DNA from DNase digestion at a 10 min reaction of 0.1 unit DNase 1 in 37°C. This cationic peptide could improve the transfection particles formed between plasmid and liposome both in size and particle distribution. Such as, by using vector of pCMVGFP (4.7kb), the average size of liposome/ peptide/vector complexes are 101 ± 9.3 nm, but liposome/vector complexes are 162 ± 24.7 nm. Lipofection with cationic M9 complexes also dramatically increased marker gene's expression in highly confluent BAEC. With standard GFP assay, the total GFP gene expression level (ng/assay) could be easily increased more than 6-16 fold to levels up to 80 nm / 2-cm^2 BAEC. This cationic M9 nuclear targeting system may eventually used in other gene targeting drug deliveries, such as plasmids, antisense oligos, RNA-DNA chimeras and peptide nuclear acids.

9:15 AM NN3.3
THE MOLECULAR BASIS OF ENGINEERED *IN VITRO* HEPATOGENESIS. Erin R. Ochoa, Massachusetts General Hospital, Dept of Pathology, Boston, MA; Kohei Ogawa, Dept of Transplantation and Immunology, Faculty of Medicine, Kyoto University, Kyoto, JAPAN; Joseph W. Carlson, Harvard-MIT Division of Health Sciences and Technology, Boston, MA; Joseph P. Vacanti, Massachusetts General Hospital, Dept of Surgery, Boston, MA.

We employ *in situ* hybridization to describe a liver engineering process at the molecular level and evaluate a strategy for engineering human replacement livers. We employ 3DP^{TM} printing technology to create a biodegradable polymer scaffold controlled to the 100-micron scale. Mimicking liver vascularization, this 3-D scaffold contains an inlet "artery" which branches to smaller vessels feeding a liver "parenchyma", and an outlet "vein." Onto this scaffold we seed isolated highly proliferative hepatocytes, then culture tissue-engineered livers in a dynamic bioreactor. Eventually, vascularized tissue-engineered liver will be constructed from a human patient/s own donor hepatocytes then anastomosed to that patient/s portal vein and induced to grow at the expense of diseased native liver while maintaining integrity of liver function and avoiding transplant rejection. Underlying this strategy is the hypothesis that, given adequate culture conditions, disassociated liver cells will spontaneously reiterate *in vivo* organogenesis. The *in vivo* process has been studied by molecular biologists using *in situ* hybridization, which employs digoxygenin-labeled RNA probes to detect the presence or absence of individual genetic transcription factors in a liver at a given time. As each factor is expressed in the developing liver they activate other regulating factors whose complex interactions control hepatogenesis. We have synthesized probes with appropriate controls to detect for each of the transcription factors which are most important to hepto-embryogenesis: HNF-3β, HNF-6, mHGF, rTAT, IGF-1, SEK-1, GATA-2, Hlx, and JNK-1. For each probe twenty-five rat embryo livers are tested at each of three gestational time points. The same probes will be used for 25 tissue-engineered rat livers at each of three time points. We expect our data to show donor hepatocytes undergoing a genetic pattern regression and then a pattern reiteration, indicating that the active culture phase of the engineering process mimics *in vivo* hepato-organogenesis at the molecular level.

9:30 AM NN3.4
NOVEL POLY(ETHYLENE GLYCOL)-CONJUGATED DENDRIMER FOR BIOCOMPATIBLE, HIGH EFFICIENT, AND LOW COST DNA DELIVERY. Dan Luo, Nadya Belcheva, Ernest Han and W. Mark Saltzman, Cornell University, School of Chemical Engineering, Ithaca, NY.

Starburst polyamidoamine dendrimer (PAMAM) is a DNA delivery agent with high efficiency, when compared to other chemical transfection reagents. Partially degraded dendrimer offers even higher efficiency, presumably due to enhanced flexibility of the otherwise rigid dendrimer. Based on the current understanding of DNA delivery, we hypothesized that chemical modification of low generation dendrimer with biocompatible poly(ethylene glycol) chains would have several potential advantages: 1). Voids created on the dendrimer surface via PEGylation could increase the flexibility of dendrimers and accommodate the DNA helixes without creating strong electrostatic interactions; 2). Biocompatibility of the molecule should increase due to the reduction in generation of PAMAM and addition of PEG branches; 3). Intracellular release of DNA molecules should be more efficient due to the presence of fewer tertiary amino groups. Here, we report on the synthesis and characterization of a novel PEG-PAMAM conjugate that increases transfection up to 20 fold compared with partially degraded dendrimer controls. This extremely efficient, highly biocompatible, and low cost DNA system can be readily used in basic research laboratories and may find future clinical applications.

9:45 AM NN3.5
ELECTROSTATIC SELF-ASSEMBLY OF ACTIN-MEMBRANE TUBULAR CAPSULES. Gerard C.L. Wong, University of Illinois at Urbana-Champaign, Materials Dept, Physics Dept; Jay X. Tang, Indiana University, Physics Dept; Youli Li, Alison Lin, University of California at Santa Barbara, MRL; Paul Janmey, University of Pennsylvannia, Institute for Medicine and Engineering; Cyrus R. Safinya, University of California at Santa Barbara, Materials Dept, Physics Dept, Biochemistry and Molecular Biology Program.

We describe a distinct new type of spontaneous hierarchical self assembly comprised of cytoskeletal filamental-actin (F-actin), a highly charged polyelectrolyte, and cationic lipid membranes, using entropically modulated electrostatic interactions. Unexpectedly, on the mesoscopic length scale, confocal microscopy reveals a ribbon-like tubule structure which connect to form a network of tubules on the macroscopic >100 micron scale. Within the tubules, on the 0.5 to 50 nanometer length scale, x-ray diffraction reveals an unusual structure consisting of osmotically swollen stacks of composite membranes with no direct analog in simple amphiphilic systems. The composite membrane is comprised of three layers, a middle lipid bilayer sandwiched between two layers of actin, and is reminiscent of multi-layered bacterial cell walls which exist far from equilibrium. Electron microscopy reveals that the actin layer consists of laterally locked F-actin filaments forming an anisotropic 2-dimensional tethered crystal which appears to be the origin of the tubule formation. The open, nested structure of these self-assembled rigid-walled tubules suggests possible applications in drug delivery. [1] G.C.L. Wong, J.X. Tang, A. Lin, Y. Li, P.A. Janmey, and C.R. Safinya, SCIENCE, in press.

10:30 AM *NN3.6
POLYMERIC CONTROLLED DELIVERY FOR GENE DELIVERY AND IMMUNOTHERAPY. Kam W. Leong, Dept of Biomedical Engr, Johns Hopkins School of Medicine, Baltimore, MD.

Non-viral vectors are increasingly being proposed as alternatives to viral vectors for in vivo gene transfer because of their potential advantages in addressing the pharmaceutical issues of applying gene as a drug. We have studied the complexation of DNA with natural or synthetic polycations in forming nanospheres. In addition to benefits common to other non-viral gene delivery systems of protecting the DNA from nuclease degradation and allowing active targeting, characteristics unique to these biodegradable DNA nanospheres include co-encapsulation of bioactive agents and sustained release of the DNA. Positive gene transfer has been observed in vivo in the lung, muscle, and gastrointestinal tissues in animal models. While the transfection efficiency of these DNA nanospheres remain low, their application in DNA vaccination, where high antigen expression may not be required, is promising because of their ability to encapsulate and deliver cytokines in a local and sustained manner to stimulate the infiltrating immune cells.

11:00 AM NN3.7
CONTROLLED RELEASE OF BONE GROWTH FACTORS FROM INJECTABLE BIODEGRADABLE POLYMER SCAFFOLDS FOR BONE TISSUE ENGINEERING. Elizabeth L. Hedberg and Antonios G. Mikos, Rice University, Department of Bioengineering, Houston, TX.

We seek to develop injectable, in situ polymerizable, biodegradable materials for treating skeletal defects with guided bone regeneration. At a minimum, the biomaterial should serve as a scaffold for cell adhesion and migration. Regeneration may be enhanced, however, through the incorporation of bioactive molecules. To that end, the injectable polyester poly(propylene fumarate) (PPF) was loaded with poly(lactic-co-glycolic acid) (PLGA) microparticles carrying FITC-Dextran and then crosslinked with N-vinyl pyrrolidinone in the presence of a benzoyl peroxide as an initiator and sodium chloride (NaCl) as a leachable porogen. The encapsulation of the growth factor in microparticles was necessary to minimize denaturing of the growth factor during scaffold crosslinking. The FITC-Dextran is used as a co-factor to assist in the release of the growth factor. Modulation of the release of FITC-Dextran will in turn lead to modulation of the release of the growth factor. PLGA microparticles were incorporated into composites of varying NaCl weight percents and the effects on FITC-Dextran release kinetics was determined in vitro for cylinders of diameter 6.5 mm and height 13.0 mm. FITC-Dextran was incorporated into the biodegradable microparticles at 10 mg FITC-Dextran/1 mg microparticles; PLGA microparticles were incorporated into the composites at 40 mg microparticles/g PPF. The FITC-Dextran loaded microparticles alone exhibited a large initial burst effect, while the composite materials displayed a smaller burst effect and a longer linear region of release. At day 1, 43.81.8%, 2.980.5%, and 8.00.7% of loaded FITC-Dextran was released into pH 7.4 phosphate buffered saline from the microparticles, the 50 wt% NaCl, and the 70 wt% NaCl composites, respectively. By day 28, 90.96.9%, 12.71.7%, and 34.40.4% of loaded FITC-Dextran was released. Our results demonstrate that PLGA microparticles can successfully be added to PPF composites and that the release kinetics of FITC-Dextran can be systematically manipulated through alterations of the composite material properties.

11:15 AM NN3.8
AN AQUEOUS SOL-GEL PROCESS FOR ENCAPSULATION OF PROTEINS AND CELLS FOR BIOSENSOR APPLICATIONS. Anup K. Singh, Alok K. Gupta, Sandia National Laboratory, Livermore, CA; Ashok Mulchandani, Dept. of Chemical and Environmental Engineering, Univ. of California, Riverside, CA; Rimple B. Bhatia, Carol S. Ashley, C. Jeffrey Brinker, Univ. of New Mexico/Center for Microengineered Materials, Albuquerque, NM and Sandia National Laboratory, Albuquerque, NM.

Porous silicate materials made by low temperature sol-gel process are promising host matrices for encapsulation of biomolecules. Their mechanical strength, chemical inertness, hydrophilic nature, and above all, their optical transparency makes them an exciting platform for development of biosensors. To date, researchers have focussed on sol-gel routes using alkoxides such as tetramethyl orthosilicate (TMOS) and tetraethyl orthosilicate (TEOS) for encapsulation of biomolecules. These routes lead to formation of alcohol as a byproduct that can have detrimental effect on the activity of entrapped biomolecules. We have developed a novel sol-gel process to encapsulate biological molecules (such as enzymes, antibodies and cells) that uses neutral pH, room temperature, and does not generate alcohol as a byproduct. The process uses sodium silicate as precursor and is carried out in two steps- preparation of a low pH silicate sol followed by gelation at neutral pH in a buffer containing biomolecules. Two enzymes widely used in biosensing applications- horseradish peroxidase (HRP) and glucose-6-phosphate dehydrogenase (G6PDH), were used to prepare enzyme-doped silica monoliths to investigate the effect of silica as host matrix on enzyme kinetics. We also developed a novel homogeneous immunoassay for 2,4,6-trinitrotoluene (TNT), and encapsulated the immunoassay reagents in sol-gel matrices to produce dispersible biosensors for the detection of TNT. Using the sol-gel material doped with immunoassay reagents, TNT at low ppm levels in water could be detected. We also report encapsulation of E. Coli cells expressing the enzyme organophosphorous hydrolase (OPH) on the cell surface in sol-gel matrices. The cell-doped sol-gel material can be used to develop biosensors for detection of organophosphorous compounds such as insecticides and chemical warfare agents.

11:30 AM *NN3.9
ANALYSIS OF ELECTROSTATIC EFFECTS ON THE SUCCESS OF RETROVIRAL-MEDIATED GENE DELIVERY. Howard E. Davis, Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA; Jeffrey R. Morgan, Martin L. Yarmush, Center for Engineering in Medicine and Surgical Services, Massachusetts General Hospital, Harvard Medical School and Shriners Burns Hospital, Boston, MA.

Recombinant retroviruses are a commonly used gene delivery tool in human gene therapy clinical trials as they permanently integrate the therapeutic DNA into the genome of the target cell. Despite their widespread use, however, retroviral gene transfer efficiencies remain disappointingly low. In the years since their initial development as vectors, it has become clear that the physicochemical properties of the recombinant retrovirus, as well as the properties of the target tissue, have a major impact on the success of gene transfer. In particular, the interaction between the native negative charge present on the vector and target cell membranes results in a significant electrostatic barrier which must be overcome in order for the transduction process to begin. Using a recently developed retrovirus adsorption assay, we have demonstrated that this electrostatic interaction is the dominant interaction during the initial steps of transduction. We have also established that cationic polymers enhance adsorption, and ostensibly transduction, by neutralizing this electrostatic barrier. Interestingly, we also found that this enhanced adsorption could be demonstrated even in the absence of a cellular receptor for the virus, suggesting that the first step of transduction does not involve binding of the virus to currently identified receptors, but is either a non-specific adsorption process or involves binding to an, as of yet, uncharacterized class of receptor. Based upon our findings, we propose a new physical model of retrovirus adsorption, which is diffusion-limited, receptor and tropism independent, and modulable by positively charged compounds. Our findings may have significant implications for other gene and drug delivery systems which interact directly with target tissues at the level of the cell membrane.

SESSION NN4: PHASE SEPARATION AND CHARACTERIZATION OF DELIVERY SYSTEMS
Chair: Balaji Narasimhan
Tuesday Afternoon, November 28, 2000
Republic A (Sheraton)

1:30 PM *NN4.1
THE DYNAMICS OF PHASE INVERSION RELATED TO INJECTABLE DRUG DELIVERY. Anthony McHugh, Univ of Illinois, Dept of Chemical Engineering, Urbana, IL.

An alternative to the use of preformed polymeric membranes for controlled delivery of peptide and protein therapies is that of polymer solution injection. In this case, protein particles are suspended in a solution of a biodegradable polymer in a bio-compatible solvent and injected subcutaneously. Contact with the aqueous-based physiologic surroundings causes liquid de-mixing and gelation of the polymer solution (phase inversion), thereby forming the membrane carrier in-vivo, simultaneously with the release of the entrapped drug. The dynamics of the membrane formation reflect the thermodynamic and mass transfer interactions in the system and these, in turn, profoundly influence the drug release characteristics. Selection of solution formulations for optimal release profiles thus requires an understanding of the role of phase inversion in the process. This talk will review recent studies that are addressing these issues. We employ dark ground imaging to visualize the phase inversion dynamics of several injectable delivery systems. These measurements are related to in-vitro drug release profiles obtained using standard techniques and, together, provide fundamental insights on the role of the key variables affecting the process. The latter include the polymer type and molecular weight, solvent quality and water miscibility, as well as the presence of "bath-side" additives. Depending on the phase inversion kinetics, protein particle densification can also have a positive effect on the release characteristics. Systematic variation of the solvent quality provides an efficient means to control release rates. Both in-vitro and in-vivo studies have demonstrated that extended-release profiles and significantly reduced bursts result when the aqueous affinity of the depot solvent is reduced. The uniform gel morphology

that results lacks the highly porous structure characteristic of rapid precipitation systems that also exhibit a strong burst effect. This suggests that optimal release profiles result from uniform diffusion of the protein through a homogeneously formed polymer matrix.

2:00 PM NN4.2
SURFACE ERODIBLE POLYANHYDRIDE COPOLYMERS AND BLENDS FOR DRUG DELIVERY APPLICATIONS. Elizabeth Shen, Brianne Dziadul, Mee-Kyung Lim, Balaji Narsimhan.

Biodegradable controlled release systems hold significant advantages over other controlled release systems since the need to surgically remove the device is obviated. An understanding of the complex relationship between the polymer microstructure and drug distribution/release would pave the way for a systematic engineering approach to designing controlled drug delivery systems. It is our hypothesis that drugs thermodynamically partition themselves into regions of similar hydrophobicity when loaded into microphase separated heterogeneous polymers. We synthesized copolymers and blends of polyanhydrides based on 1,6-bis-p-carboxyphenoxy hexane (CPH) and sebacic acid (SA). We examined the polymer microstructure and the effects of loading these polymers with two model drugs-hydrophilic brilliant blue and hydrophobic p-nitroaniline. Morphological characterization was carried out using differential scanning calorimetry (DSC) and wide-angle X-ray diffraction (WAXD). These methods showed that brilliant blue had no effect on the crystalline structure of the polymer while p-nitroaniline disrupted the crystalline structure, causing loading-dependent melting point depression. Surface microstructure was examined using atomic force microscopy (AFM) and small angle X-ray scattering (SAXS). These techniques provided evidence of microphase separation in copolymer compositions exceeding 75% of one component. Kinetics studies were carried out to obtain drug and monomer release profiles. Significant correlations between the polymer microstructure and the drug/monomer release kinetics emerge, depending upon the copolymer composition and the drug hydrophobicity. By examining fundamental monomer-monomer and polymer-drug interactions, we hope to better predict and design controlled drug delivery systems for specific applications.

2:15 PM NN4.3
DIRECT MEASUREMENT OF INTERACTIONS BETWEEN TETHERED PEG CHAINS AND ADSORBED MUCIN LAYERS BY SURFACE FORCE APPARATUS. Yanbin Huang, School of Chemical Engineering, Purdue University, West Lafayette, IN; Nadezhda Efremova, Deborah E. Leckband, Department of Chemical Engineering, University of Illinois, Urbana, IL; Nicholas A. Peppas, School of Chemical Engineering, Purdue University, West Lafayette, IN.

We used surface force apparatus (SFA) to make direct force measurement between tethered poly(ethylene glycol) (PEG) chains and an adsorbed mucin layer. The mucins were adsorbed on the naked mica surface, while the PEG chains were tethered on a lipid bilayer. Force measurements were made in pH 7.2 and 4.0 buffer solutions, and the results showed that there was no notable attraction between the two surfaces. The in-situ mucin adsorption experiments were done at pH 7.2 by using surface plasmon resonance (SPR) measurements, and it was found that there was no permanent binding of mucins on the PEG-tethered surface. However, the adsorption experiment of mucins on a self-assembled PEG monolayers in pH 2.0 buffer solutions showed permanent binding, and hence suggested there were some attractions between PEG and mucins at this low pH condition. This work showed that SFA could provide information of molecular interaction between polymers and simulated mucus layers, which is essential for the design of new mucoadhesive drug delivery systems.

2:30 PM *NN4.4
SMALL MOLECULE DIFFUSION AND SOLUBILITY IN ADHESIVES USED FOR TRANSDERMAL DRUG DELIVERY: INFRARED-ATTENUATED TOTAL REFLECTANCE (IR-ATR) STUDIES. Adam S. Cantor, 3M Drug Delivery Systems Division, St. Paul, MN.

A key factor in designing a drug-in-adhesive transdermal drug delivery system is to understand the rate at which the drug and small-molecule excipients can diffuse in the adhesive matrix. The solubility of these components in the adhesive matrix is also of great importance. Results will be presented discussing the use of infrared-attenuated total reflectance (IR-ATR) spectroscopy as a method to measure both diffusion and solubility of small molecules in adhesives. In this method, the donor layer is either a doped adhesive or a free liquid that is placed in contact with a receptor layer which is an undoped adhesive in contact with an IR-ATR crystal. The IR-ATR crystal detects as a function of time any molecules that diffuse from the donor layer into and through the receptor layer. Examples will be discussed of several different experiments that can be performed with this technique. Using a doped adhesive layer, diffusion coefficients have been determined for testosterone and terpineol in an isooctyl acrylate based adhesive. Diffusion and solubility of liquids in several adhesives has been determined using the experiment where a free liquid is used as the donor. Diffusion from a doped layer containing dispersed, as well as dissolved solute has also been performed with testosterone to simultaneously determine solubility and diffusion coefficient of a solid solute. Finally diffusion from a doped layer of one adhesive to an undoped layer of a different adhesive can be used as a partition measurement. The parameters that can be extracted from each of these experiments, as well as the limitations of each type of experiment will be discussed.

3:15 PM NN4.5
PHASE SEPARATION IN POLY(PSUEDO AMINO ACID) - PEG BLENDS AND COPOLYMERS. M. Libera, Stevens Institute of Technology, Hoboken, NJ; M. Jaffe, Medical Device Concept Laboratory, Newark, NJ; J. Kohn, Rutgers University, Piscataway, NJ.

The hydrophilic properties of resorbable polymers for drug-delivery and other biomaterials applications are often controlled by combining poly(ethylene glycol) (PEG) with a more hydrophobic polymer phase. Relatively little is known about the local morphology and nature of phase separation in such PEG-modified systems, however. This research studies the development of phase-separated morphology in blends and random-multiblock copolymers of a tyrosine-based poly(pseudo amino acid) [poly(DTE carbonate)] and PEG 1000. This system exhibits attractive biocompatability, strength and modulus, and resorption behavior which can be controlled by main-chain and pendant-chain chemistries. The nature of phase separation was studied at high spatial resolution by transmission electron microscopy (TEM) using solvent-cast thin films. The various films display qualitatively different phase-separation behaviors with morphological features having characteristic length scales ranging from 10nm to 1mm. These length scales are comparable to those characteristic of proteins and of cells and the nature of phase separation can be anticipated to influence both protein and cell adhesion phenomena. Co-continuous morphologies may be possible in blends of appropriate composition and using various processing strategies. For high-PEG concentrations, cryo-electron microscopy shows that these systems can form water soluble micelles with characteristic length scales of order 50 nm depending on polymer molecular weight.

3:30 PM NN4.6
BIMOLECULAR MATERIALS CHARACTERIZATION USING STATIC AND DYNAMIC LASER LIGHT SCATTERING DETECTION TECHNOLOGIES. John P. Helfrich, Life Science Group, Precision Detectors, Inc., Franklin, MA.

Rugged characterization assays for new therapeutic and diagnostic biomaterials (proteins, antibodies and polymeric formulations) is a fundamental task for the successful submission of data for regulatory approvals and proper quality control documentation. A key component of this data is the accurate assessment of the molecular weight and/or molecular weight distribution or aggregation state. Most bio-macromolecules can aggregate as a function of temperature, pH, ionic strength and concentration. Even small amounts of aggregates (dimer, trimer, etc.) can be significant as they cause conformational shifts in the molecular structure that can alter the intended function as an effective therapeutic or diagnostic agent. Monitoring and understanding these effects are fundamental for process development and quality control requirements.
Laser light scattering (LLS) detectors have been used for over a decade to determine the molecular weight characteristics of industrial polymers. Recent innovations in modern high speed electronic components such as high performance diode lasers, high-speed digital signal processors and modern avalanche photodiode detectors has lead to the evolution of a new combined *static and dynamic* laser light scattering detector. This detector has a 10 uL flow cell design and is capable of characterizing both molecular weight *and* size for biomolecules when coupled to modern HPLC/SEC instruments. This detector and associated software provides: absolute molecular weight data for each eluting component in the range of 1 kD to 10 million daltons and hydrodynamic radius (R_h) measurements for biomolecules in the range of 1 nm to 1000 nm.
This paper will outline the design and applications of this unique detection system for "well-characterized" biomolecules and their controlled release formulations.

3:45 PM NN4.7
CHARACTERIZATION AND BIOCOMPATIBILTY STUDIES OF LAYER-BY-LAYER SELF-ASSEMBLED HUMIC ACID/Fe^{3} FILMS. Izabela Galeska, Fotios Papadimitrakopoulos, Dept of Chemistry, Polymer Sci Program, Nanomaterials Optoelectronics Lab, Inst of Matls Sci, Univ of Connecticut, Storrs; Tammy Hickey, Francis Moussy, Ctr for Biomaterials & Surgical Research Center, Univ of Connecticut Health Center, Farmington, CT.

A stable, soft, semipermeable and non-inflammatory membrane is a prerequisite for the development of an implantable glucose biosensor for continuous pain free monitoring of glucose levels in vivo. Humic acids have been reported to have therapeutically relevant in vivo characteristics such us antiviral and anti-inflammatory. This encouraged us to investigate in vivo compatibility of Humic acid based multilayered films as a potential membrane material for the implantable glucose sensors. Humic Acids (HA) are naturally occurring biopolymers found in soils, sediments, water and some plants like peat and tobacco. Electrostatic layer by layer self-assembly technique of HA with oppositely charged ferric ions was utilized to grow films, which could potentially be used as the outer semi-permeable, protective layer for the glucose sensor. The growth characteristics of these assemblies exhibits strong dependence on the pH and the ionic strength of HA solution and can be correlated with the degree of ionization of carboxyl groups and the neutralization induced surface spreading. Quartz Crystal Microbalance (QCM) and ellipsometric studies have shown repeatable, stepwise increase in mass and in film thickness during the self-assembly. Importantly these films exhibit reversible swelling in water and have a shear modulus of about 50 MPa which implies stability on implantation. The biocompatibilty of these films were studied by implanting HA/Fe^3 coated silastic tubing in rats and comparing the tissue response to a medical grade silastic tubing under the same conditions. The tissue response of these films compares favorably with the silastic tubing after 4 weeks of implantation.

4:00 PM NN4.8
A NOVEL POLYETHYLENE DEPOT DEVICE FOR THE STUDY OF PLGA NANOSPHERES IN VIVO. Maryellen Sandor, Joshua H. Harris, Edith Mathiowitz, Brown University, Dept of Molecular Pharmacology, Physiology, and Biotechnology, Providence, RI.

Polymer nanospheres are difficult to characterize in vivo due to their tendency to degrade, migrate, and be endocytosed. A novel polyethylene mesh device which contains nanospheres in vivo allowed for retrieval of degraded products from rats. Pouches containing PLGA nanospheres were implanted intramuscularly, subcutaneously, and intraperitoneally for 3 days, 1, 2, or 4 weeks. Explants were photographed, analyzed by GPC, and measured for mass loss. Devices tested in vitro were compared to naked nanospheres to determine device effect on degradation rates. In vitro, encased and naked nanospheres exhibited very similar degradation profiles, indicating no interference by the device itself. Devices implanted in vivo behaved differently from those tested in vitro for the first 2 weeks, after which in vivo and in vitro results converged. In vitro, Mw decreased immediately to 56.17% and 28.67% of the original value by 72 hours and 1 week, respectively. In vivo, the decrease in Mw lagged initially, with no change from the original value at 72 hours for subcutaneous and intraperitoneal implants, and a decrease to 64.80% and 84.19% for those implanted 1 week. Conversely, samples implanted subcutaneously and intraperitoneally began to lose mass immediately, dropping to 76.00% and 64.50% of the original mass by 1 week, while those tested in vitro had only decreased to 94.00% and did not begin to lose mass appreciably until after 1 week. Intramuscular implants behaved more like in vitro samples decreasing in Mw immediately (79.37% and 57.10% by 3 days and 1 week) and lagging in mass loss (98.50% and 80.00% by 3 days and 1 week). These results suggest that mass loss, which is usually preceeded by and dependent on Mw loss in vitro, may initially be directly due to enzymatic, rather than hydrolytic, degradation subcutaneously and intraperitoneally. Intramuscular implants may undergo a different mechanism of degradation.

4:15 PM NN4.9
ADHESION OF PRESSURE SENSITIVE ADHESIVES WITH APPLICATIONS IN TRANSDERMAL DRUG DELIVERY. Marc B. Taub and Reinhold H. Dauskardt, Stanford University, Department of Materials Science and Engineering, Stanford, CA.

The development and implementation of successful transdermal devices for drug delivery requires an understanding of the adhesion occurring between the device and the soft dermal layer. The trend towards increasingly complex and novel patch designs further necessitates the development of a systematic approach to quantify this adhesion. Pressure sensitive adhesives (PSAs) are used as the adhesive due to their desirable properties of good initial and long-term adhesion, clean removability, and skin and drug compatibility. In addition, their highly viscoelastic properties are necessary prerequisites for attachment to soft tissue. However, the adhesion of PSAs is not well understood with almost no reproducible test methods or quantitative adhesion data. This study utilizes a mechanics approach to quantify the adhesive properties of representative PSAs. Delamination of PSAs is accompanied by cavitation in the PSA and the formation of an extensive cohesive zone behind the debond tip. The presence of such large-scale bridging provides significant energy dissipation and increased resistance to delamination. The strain energy release rate (G) during debonding of a cantilever-beam sample, containing at its midline a thin layer of PSA, was utilized to quantify the adhesion of the PSA. The analysis accounts for both the work of adhesion as well as the viscoelastic constitutive behavior of the soft adhesive layer. Effects of adhesive layer thickness, strain rate, physiological environment, and permeation-enhancement additions will be discussed.

4:30 PM NN4.10
BIOENGINEERING 3D NANOSTRUCTURES CHARACTERIZATION. Alexandra G. Bezrukova, St. Petersburg State Technical Univ, St. Petersburg, RUSSIA.

Static and dynamic light scattering can provide further progress in on-line control of complex 3D disperse systems such as liposomes carrying various substances (enzymes, viruses, etc.), blood substitutes and others bioengineering nanostructures. These methods are also compatible with the nondestructive analysis of disperse systems by other optical methods: refractometry, absorbancy and fluorescence. Our reseach has investigated different disperse systems: liposomes, blood substitutes, proteins, nucleoproteins, viruses, lipoproteids, lipid emulsions, etc. and mixtures - liposomes and viruses, blood substitutes with blood serum, etc. by static light scattering (integral and differential, unpolarised and polarised) and dynamic light scattering. For the solution of inverse physical problem of static light scattering the fitting method with approximation of particles as homogeneous spheres, core-shell structured spheres, oblate and prolate ellipsoids of rotation and regularization procedure for inverse problem of dynamic light scattering have been applied. By optical methods it is possible to determine parameters of disperse systems state (mean equivalent diameter and number of particles, mean refractive index and mass of disperse phase, number and mass distributions) and parameters of particles structure: form and thikness of shell.

4:45 PM NN4.11
THERMOMECHANICAL PROPERTIES OF GELS: STATICS AND DYNAMICS. M. Naci Inci, Burak Erman, Faculty of Engineering and Natural Sciences, Sabanci University, Orhanl, Istanbul, TURKEY; Selda Durmaz, Oguz Okay, TUBITAK Marmara Research Center, Kocaeli, TURKEY.

Highly swollen gels used in drug delivery technology and related areas are strongly controlled by their thermo-mechanical properties. Among these is the elastic modulus of the gel as a semi-open system, where the solvent moves in and out of the gel depending on the state of stress and environmental thermodynamic conditions. In the present study, elastic moduli of cross-linked swollen poly(isobutylene) spheres and films are studied by monitoring deformations under stress, using an optical microscope. The gels are prepared by suspension polymerization. Stress relaxation in beads of about 1-2 mm in diameter is studied by uniaxially compressing a bead between two walls and characterizing their instantaneous moduli as a function of time. A simple constitutive equation is proposed that relates the amount of solvent in a compressed gel to time, stress and thermodynamic and structural variables.

SESSION NN5: POSTER SESSION
BIOMATERIALS FOR DRUG DELIVERY
Chair: Edith Mathiowitz
Tuesday Evening, November 28, 2000
8:00 PM
Exhibition Hall D (Hynes)

NN5.1
MORPHOLOGY AND RELEASE PROFILE OF ACTIVE LOADED POLYHYDROXYALKANOATE MICROPARTICLES. Teresa Eligio, Ruben Sanchez, Polymer Section, Advanced Material Laboratory, North Fluminense State University, UENF, Campos, R.J., BRAZIL.

The poly-3-hydroxyalkanoates (PHAs) are biocompatible and biodegradable polymers family suitable for biomedical purposes. In this work are presented a preliminary study to establish a morphologic and release profile difference between two family members, poly-3-hydroxybutyrate, PHB, a high cristallinity polymer, and the poly-3-hydroxyoctanoate, PHO, amorphous material in order to encapsulate two different loaded; a low molecular weight and relative high molecular weight drugs, both hydrophilic materials. Two kinds of polymeric devices were obtained: reservoirs (capsules) and monolithic (matrix) produced by different techniques; oil in water emulsion (o-w), water-oil-water double emulsion (w-o-w) and spray drier. The external and internal microparticles morphology studied by Scanning Electronic (SEM) and Transmission Electronic Microscopy (TEM) techniques showed differences in the internal distribution of the drug on the microparticles associated to encapsulation techniques. The external texture, independent of preparation techniques, indicates

a rough surface morphology , sphericity and diameters ranging between 5-10 mm. The porosity and tortuosity of the polymeric devices had a direct impact on the release characteristics. In general, for the emulsion techniques the release pattern is zero or first order and for the sprier drier techniques the Higuchi "t Ω" dependence were observed.

NN5.2
A NOVEL CATIONIC CARBOHYDRATE-BASED LIPID FOR GENE DELIVERY. Keeana S. Sajadi, Geoffrey S. Hird, Mark W. Grinstaff, Duke Univ, Dept of Chemistry, Durham, NC.

Cationic lipid based gene delivery systems such as DOTMA (N[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride)/ DOPE (dioleylphosphatidylethanolamine) mixtures have been used for both in vivo and in vitro transfection of nucleic acids. The basic components of a cationic lipid are the hydrophobic lipid group that aids in interactions with the cell membrane, the positively charged headgroup which facilitates condensation of the negatively charged plasmid and a linker group between the two which is important for both biodegradability and chemical stability of the compound. Currently there is an intensive research effort in optimizing the transfection efficiency by altering the cationic head group, the hydrophobic lipid group, and the linker. We have synthesized and characterized a novel cationic lipid containing ribose as the biocompatible and biodegradable linker (1-methoxy-2,3-dilauroyl-ribo-5-lysine). The ability of these novel molecules to transfect DNA in vitro is underway.

NN5.3
A QUANTITATIVE TECHNIQUE FOR DETERMINING THE EXTENT OF POLYMER UPTAKE. Chris Thanos, Edith Mathiowitz, Brown University, Department of Molecular Pharmacology, Physiology, and Biotechnology, Providence, RI.

Microspheres of varying size cross the mucosal lining of the small intestine and enter systemic and lymphatic circulation within less than an hour. Although the exact mechanism has yet to be elucidated, it has been speculated that uptake can occur via the gut-associated lymphoid tissue (GALT), across the apical membrane of absorptive epithelium, and through paracellular routes. Previous work has concentrated on histological analysis of tissue including confocal microscopy and TEM, however the results have served primarily as qualitative characterization. In this study, biodegradable polymers, including PLGA, FASA, and PCL were blended with a polystyrene-linked far-red fluorescent dye into particles ranging from 200 nm to 5 microns in diameter and incubated in rabbit jejunum for 1 hour. Following tissue excision and fixation for histology, tissue was vigorously flushed to remove particles on the surface and aliquoted for fluorescence analysis. This tissue was lyophilized to obtain dry weights, incubated in 8% KOH for 24 hours at 60C, and put through a series of microcentrifugal separation techniques at various molecular weight membrane cutoffs to obtain a layer of polystyrene-linked dye. KOH digests the tissue components along with the polyanhydride and polyester microspheres used in this study, hence the final component in the extraction includes only the polystyrene and fluorescent dye which is easily quantified without background interference from cellular and polymer elements. This material is resuspended in aqueous solution and fluorometric analysis is used comparing the samples to standards prepared from stock beads in aqueous suspension. This method can be used to quantify uptake provided the loading of dye is known and the fluorescent polystyrene beads are uniformly labeled, and was used to validate the results of confocal experiments comparing the aforementioned biodegradable polymers.

NN5.4
HIGH-PRESSURE INDUCED LIQUID CRYSTAL PHASES MAINTAINED IN NON-MESOGENIC POLYMERS AT AMBIENT TEMPERATURE AND PRESSURE. Edwin Edwards, Edith Mathiowitz, Dept of Molecular Pharmocology, Physiology and Biomaterials, Brown University, Providence RI.

liquids (no long-range order), and crystals with long-range three-dimensional order. These phases, called mesophases, are created by unique rigid molecular structures called mesogens, which are generally rod-shaped or disk like. These mesogens can be individual low molecular weight molecules or part of repeats in a polymer chain (polymer liquid crystal). The reigning philosophy has been that liquid crystal mesophases can only occur in mesogenic materials however recent discoveries indicate the potential of inducing these phases in non-mesogenic materials. The phase is induced by subjecting the material to a combination of temperature and pressure conditions and appears to be maintained at ambient conditions for extended periods of time. However, when the material is reheated beyond a critical temperature the induced phase is removed and the material returns to its native state. These novel liquid crystal materials could be used as safer hip replacement polymers or in drug delivery devices.

NN5.5
SEGMENTED POLYURETHANES FOR CONTROLLED DRUG DELIVERY. Erkesh O. Batyrbekov, Rinat M. Iskakov, Bulat A. Zhubanov, Institute for Chemical Sciences. Almaty, KAZAKSTAN.

The segmented polyurethanes with different content of soft and hard segments have been studied as drug carriers for controlled delivery application. Biodegradable polyurethanes were obtained by means of a two step procedure using different polyetherglycoles, diisocyanates and branching agents. Drugs such as antituberculous agents izoniazid and ethionamide, antibiotics kanamycin and rifampicin, local anaestetics kazkain and trimekain were incorporated into polymer by the stage of polyurethane synthesis or adsorbtion from aqueous solutions.
The influence of different factors such as the chemical structure and loading of drugs, the nature of polyethers, the supramolecular structure and morphology of polyurethanes on the drug release rate were investigated. It was shown that the microdomain structure of polyurethanes play significant role for regulation of drug release.
The successful application of Polyurethane Drug Delivery Systems for the treatment of tuberculosis, esophagus burns, glaucoma and some dental diseases was shown.

NN5.6
Abstract Withdrawn.

NN5.7
A POLYCARBONATE OF GLYCEROL. William C. Ray III and Mark W. Grinstaff, Duke Univ, Depts of Chemistry and Ophthalmology, Durham, NC.

New biopolymers are needed to meet the current challenges in orthopaedics, dentistry, cardiovascular surgery, and ophthalmology. Polycarbonates are one class of polymer being investigated, and polymers of 4-hydroxymethyl-1,3-dioxolan-2-one are known. However, no polymers of its 6-membered cyclic isomer, 5-hydroxy-1,3-dioxan-2-one, have been made. The cyclic carbonate 5-benzyloxy-1,3-dioxan-2-one has been synthesized from glycerol and polymerized. Following cleavage of the benzyl group by catalytic hydrogenolysis, the resulting polymer is a novel polycarbonate of glycerol. The benzyl protected and free-hydroxy polymers have been characterized by GPC, NMR, IR, DSC, and contact angle. This glycerol polycarbonate is a potentially useful biomaterial since it is expected to hydrolyze in vivo to glycerol and carbon dioxide. The pendant hydroxyl groups of poly(5-hydroxy-1,3-dioxan-2-one) also provide a side chain for post-polymerization modification (i.e. attachment of additional functional molecules).

NN5.8
BIODENDRIMERS FOR DRUG DELIVERY. Michael Carnahan, Mark W. Grinstaff, Duke Univ, Dept of Chemistry and Ophthalmology, Durham, NC.

Dendrimers are monodisperse spherical macromolecules in which all bonds converge to a focal point or core. In contrast to linear polymers, dendrimers possess a large numbers of controllable terminal functionalities and exhibit low viscosities, high solubility, miscibility, and adhesive properties. Consequently, dendrimers display a variety of physical properties desirable for industrial and biomedical applications. For example, dendrimers have shown significant potential as drug delivery agents by physical entrapment or covalent attachment of drugs. We are synthesizing hydrolyzable aliphatic polyester biodendrimers containing glycerol and succinic acid. These biodendrimers contain a tetra-functional core, synthesized from one succinic acid and two glycerol units, and an AB2 monomer, synthesized from one succinic acid and one glycerol unit. To date, the G1 biodendrimer, [G1]-PGlSu, has been synthesized and fully characterized by 1H-NMR, 13C-NMR, IR, MS, and elemental analysis. Further generations of this biodendrimer will be synthesized and biocompatibility will be determined with each subsequent generation. Drug encapsulation studies are ongoing to assess the efficacy of this novel biocompatible macromolecule for drug delivery applications.

NN5.9
'CARBOHYDROSOMES': A NEW CLASS OF POTENTIAL DRUG DELIVERY VESICLES. Geoffrey S. Hird, Duke University, Dept of Chemistry, Durham, NC; Thomas J. McIntosh, Duke University Medical Center, Dept of Cell Biology, Durham, NC; Mark W. Grinstaff, Duke University, Dept of Chemistry, Durham, NC.

Spherical self-closed structures called liposomes self-assemble from conventional glycerol based phospholipids, such as phosphatidylcholines. Naturally occurring phosphoglycerides are composed of a glycerol backbone, two hydrophobic hydrocarbon chains and a polar head group, that is often a choline. Current research in this field focuses on altering the head group and hydrophobic tails to attain desired physical properties of liposomes for drug delivery. We are

manipulating the backbone as a new means to optimize the chemical and physical properties for biotechnological applications and to assess supramolecular structure formation. Selecting ribose as the new backbone, we have termed these novel carbohydrate three-dimensional supramolecular structures "carbohydrosome". The ribose backbone analog of dilauroylphosphatidyl choline (DLPC), 1-methoxy-2,3-dilauroyl-ribo-5-phosphocholine (DLRPC) as well as the ribose analog of dimyristoylphosphatidyl choline (DMPC), 1-methoxy-2,3-dimyristoyl-ribo-5-phosphocholine (DMRPC) were synthesized. The resulting carbohydrosomes were characterized by optical microscopy, modulated differential scanning calorimetry (MDSC), and X-ray diffraction. Mixtures of cholesterol and DLRPC were extruded and 200 nm liposomes containing the water soluble dye 5(6)-carboxyflourescein were formed. The synthesis of DLRPC, its ability to form stable supramolecular structures, and the ability to derivatize DLRPC provides a unique opportunity to synthesize and engineer vesicles for specific biomedical applications.

NN5.10
SYNTHESIS AND CHARACTERIZATION OF CORALLINE HYDROXYAPATITE - GELATIN COMPOSITE MICROSPHERES FOR ORTHOPAEDIC APPLICATIONS. M. Sivakumar and K. Panduranga Rao, Biomaterials Laboratory, Central Leather Research Institute, Adyar, Chennai, INDIA.

Calcium phosphates in the form of hydroxyapatite (HA), have been widely used for bone implant material. These materials exhibit several problems of handling and fabrication, which can be over come by mixing with a suitable binder. Recently, a great interest has been shown on the use of composites of biodegradable polymers, such as fibrin, collagen, gelatin, chitosan and alginate, with inorganic powders, as bone fillers. Owing to their physiochemical and biological properties, calcium phosphates have recently been considered as a potential material for bone drug delivery systems. In this paper, composite microspheres of coralline hydroxyapatite (CHA) granules with gelatin, prepared by dispersion polymerization technique is reported. These composite microspheres were characterized by various techniques such as XRD, FT-IR, TGA and DSC. The particle size distribution of the composite microspheres were analyzed using particle size analyzer and the average size was found to be 16 microns. The optical micrographs clearly indicated that the microspheres are spherical and uniform in size. These CHA-gelatin composite microspheres were also loaded with antibiotic drug such as gentamicin and its in-vitro drug release profiles in phosphate buffer were evaluated.

SESSION NN6: NOVEL APPROACHES TO DRUG DELIVERY SYSTEMS
Chair: Edith Mathiowitz
Wednesday Morning, November 29, 2000
Republic A (Sheraton)

8:30 AM *NN6.1
DEVELOPMENT OF BIODEGRADABLE POLILACTOFATE MICROSPHERE (PACLIMERTM) FOR SITE SPECIFIC CANCER THERAPY. Wenbin Dang, Zhong Zhao, Stephen Dordunoo, Guilford Pharmaceuticals Inc., Baltimore, MD.

The advances in the field of controlled drug delivery have been continually fueled by the desire to improve the therapeutic effects of existing pharmaceutical substances (EAS). The key to achieve the desired improvement is to deliver the drug of an efficacious concentration directly to the site of action for a prolonged period of time so the pharmacological effects are maximized while the side effects are minimized for the drug. Biodegradable polymers such as PLGA are frequently used to engineer such controlled drug delivery systems. For certain highly insoluble chemotherapeutical agents such as paclitaxel the desired efficacious results are achieved by maintaining a continuous supply of the drug from the degrading polymer matrix. We have developed a new synthetic biodegradable polyphosphoester (polilactofate) for such drug delivery applications. Polilactofate comprises mainly of lactic acid oligomers which are separated by ethyl phosphoester bonds. One major potential advantage of polilactofate is that it is majority composed of polylactic acid which is approved by FDA and has an excellent safety record, while the interspersing ethyl phosphate groups make the final polymer more hydrophilic and degrade more evenly. The final polymer polilactofate has been proven biocompatible in various animal models. The overall degradation rate of the polymer may be further modified by varying the ratio between the lactide and the phosphate segments. The relatively rapid degradation of the polymer matrix is desired for applications such as local chemotherapy where repeated dosing of the drug is critical to the treatment. The development of a targeted cancer therapy using polilactofate PACLIMERTM micropsheres will be highlighted as an example in this presentation. PACLIMERTM micropsheres are polilactofate microspheres containing 10% paclitaxel. PACLIMERTM micropsheres has been demonstrated to release paclitaxel continuously for over three months in vivo. The synthsis, in vitro and in vivo degradation, and the degradation mechanism will be discussed. The preclinical results including the efficacy studies of PACLIMERTM micropsheres in various preclinical animal cancer models such as ovarian cancer and non-small cell lung cancer will also be presented.

9:00 AM NN6.2
A NANOTECHNOLOGICAL APPROACH TO OSMOTIC CONTROLLED LOCAL DRUG DELIVERY DEVICES. Thomas Sawitowski, Wolfgang Brandau, Alfons Fischer, Guenter Schmid, Univ of Essen, Essen, GERMANY.

Local drug delivery is up to now based on two general approaches. On the one hand a drug containing polymeric material is (chemically) dissolved in contact with body liquids. On the other hand a mechanically operating device made of a reservoir (and a catheter) is implanted in the body to deliver drugs over time. In both cases the advantage of the local delivered drugs are less side effects and low dosage needed for therapy. The disadvantage of the chemically driven polymeric devices is the low amount of drug which can be delivered and problems related to the delivery kinetics which is preferred to be of zero order with respect to time. For the built in devices their size is very often a problem. Here we report on a new technology which is based on chemically modified nanoporous membranes which enables us to vary the delivery rate easily. The basic material is an nanoporous ceramic membrane which is produced by an electro-chemically oxidation process. The pore size of those membranes can be varied between 20 nm and 250 nm while the thickness can be as big as 300 μm. The surface of those ceramic layers can be modified in order to change the surface polarity or to anchor specific moieties to the surface. This leads to a change in the interaction between the pore structure and the delivered substance. Both the pore size and the surface chemistry are the tools to control and adjust the delivery rate to the drug and the therapy. We report on results for the delivery of ^{111}In-labelled Octreotide which is a Somatostatin analogue used in cancer therapy. This oligopeptide consists of 8 amino acids linked by a disulfide bridge. For this rather small molecule we can show huge variations of the delivery rate. Changing the pore size from 50 nm to 80 nm leads to strong increase in the rate. In addition if alkyl chains of different length are chemically bound to the pore surface the deliver rate can be reduced to almost zero. In all cases a zero order time dependency of the delivery rate is observed. Because of the variable sizes those membranes can be produced we believe that implants can be built as small as possible. This opens up new ways for local drug delivery therapy like minimal invasive therapy of tumors.

9:15 AM NN6.3
DIFFUSION OF DENDRITIC POLYMERS THROUGH CONCENTRATED POLYMER SOLUTIONS. James L. Thomas, Columbia University, Departments of Chemical and Biomedical Engineering, NY.

Diffusional dynamics of polymers are very sensitive to polymer architecture. For example, Won and Lodge have shown that "arm-retraction" can be an entropically rate-limiting step for diffusion of star polymers. Unusual diffusional dynamics could facilitate the controlled release of therapeutic compounds from gels or concentrated polymer solutions with potentially novel, useful kinetics. Towards this end, we have been studying the diffusion of model dendritic polymers in concentrated solutions of "matrix" polymers, using confocal laser-scanning photobleaching and recovery. The self-similar polyamidoamine dendrimers grow exponentially in size with generation (number of synthetic cycles). High generation dendrimers (>6) are rather compact and their diffusion behavior depends only on the ratio of their hydrodynamic size to the matrix mesh size. Lower generation dendrimers are capable of partial entanglement with the matrix itself; the consequences of this entanglement will be presented.

9:30 AM NN6.4
ELECTROKINETICALLY BUILT MICRO BEAD AND CELL ARRAYS FOR DRUG DISCOVERY PRACTICE. Mihrimah Ozkan, Sadik C. Esener, Electrical and Computer Engineering Department, University of California at San Diego, San Diego, CA; Sangeeta N. Bhatia, Bioengineering Department, University of California at San Diego, San Diego, CA.

We have developed a novel electrochemical system for field assisted, fluidic assembly of objects on a microfabricated silicon substrate by means of electrical and optical addressing. The principle of our technique is the movement of charged species in solution to oppositely charged electrodes, as seen commonly in electrophoresis. Here, charged species such as beads and cells are moved electrokinetically through an aqueous solution towards a charged electrode. Micropatterning of the electrodes allows localization of charged species. This technique has been previously utilized extensively for localizing DNA. Here we first demonstrate the localization of negatively charged polystyrene

beads as model cells of various sizes (0.8-20 μm in diameter) on micropatterned substrates. In addition, light emitting diodes (50 μm diameter) and SiO_2 pucks (100 μm in diameter) have been utilized to demonstrate the general utility of this technique as a tool to interface disparate materials. Finally, 20-30 μm diameter live mammalian cells were patterned on regular arrays by means of electrical addressing. Our results indicate that cell survival was sufficient for subsequent attachment, spreading, mitosis, and passage of 3T3 fibroblasts. For applications in high-throughput phenotyping, neural stem cells were also studied. Single cell patterning was achievable on an agarose patterned, optically-transparent, semiconductor. This experiment demonstrates the feasibility of microscopic monitoring of many live cells in real-time, in parallel. This technique has applications in creation of active cellular arrays for cell biology research, drug discovery, and tissue engineering.

9:45 AM NN6.5
VITAMIN E TPGS ENCAPSULATED IN MESOPOROUS ALUMINA: A NOVEL ORAL DRUG DELIVERY SYSTEM.
Ying Ma, Kenneth J. Balkus, Jr., University of Texas at Dallas, Department of Chemistry, Richardson, TX.

Vitamin E TPGS, a modified form of the natural source of vitamin E containing a hydrophilic PEO chain, has been successfully immobilized inside of a novel mesoporous alumina using a template synthesis method. Free flowing powders were obtained, which are referred to as Al-DAM-1 (Dallas Amorphous Materials). These powders overcome the difficulty in directly handling and delivery of the sticky vitamin E TPGS. Upon exposure to simulated gastric fluid, the Al2O3 host molecular sieve is dissolved while vitamin E TPGS is released. Through this synthesis method, as much as 0.6 g of vitamin E TPGS can be immobilized into 1 gram of Al2O3. Details of the synthesis and characterization of Al-DAM-1 will be presented. Prelimilary results for using Al-DAM-1 for oral delivery of other drugs will be presented as well.

10:15 AM NN6.6
MICRONUTRIENTS AND THERAPEUTIC ELEMENT DELIVERY FROM A BIODEGRADABLE SEMICONDUCTOR: MESOPOROUS SILICON.
L.T. Canham, C.L. Reeves, P.J. Wright, T.I. Cox, Sensors and Electronics Division, DERA Malvern, UNITED KINGDOM.

The introduction of porosity at the nm scale into silicon renders the semiconductor bioactive and biodegradable (1) We describe here one area of drug delivery which aims to exploit the biodegradability, purity and high temperature processing capability of porous silicon. Many elements of the periodic table (eg Se, Cr, Mrn, Mo) are needed by the body at extremely low levels ("trace elements" or 'micro-nutrients') and yet deficiency effects are well documented. This is often simply due to diet inadequacy and because only a small and highly variable fraction of orally-ingested microminerals are absorbed. Other elements (eg Li, Au, Ag) have widespread use clinically for therapeutic purposes. We have started to investigate the use of micromachined Si tablets, doped with a range of such elements via a recently developed technique of pore impregnation and high temperature anneal. By distributing the element within the Si skeleton itself we hope to eliminate the common 'burst effect' seen with porous implants, and achieve controlled precise delivery over tunable timescales of months to years.
(1) L.T. Canham Adv. Mater. 7,1033 (1995).

10:30 AM NN6.7
CYCLODEXTRIN POLYMERS FOR ABSORPTION AND RELEASE OF ORGANIC MOLECULES.
DeQuan Li and Jason Han, Department of Chemistry and Materials Research Center, Washington State University, Pullman, WA.

Key requirements in designing nanostructured materials with abilities to absorb or release organic molecules include suitable nanocavities to dock target moelcules, optimum molecular interactions, and triggering mechanisms to alter interaction strength. A class of enzymatic compounds, cyclodextrins, have received great attention as such candidates because they have hydrophobic cavities suitable for inclusion of many organic molecules. Currently, we employ cyclo-dextrins and diisocyanates to synthesize a class of polymers with nanostructured pores. The results are a class of materials that have nanopores with great affinity to organic molecules. Indeed, cyclodextrin nanoporous polymers have such high affinity to organic molecules in an aqueous environment that they effectively scavenge even trace amount of organic molecules down to concentration levels of parts-per-billion. In this contribution, we will provide an account of our continuing effort at evaluating the absorption/release of organic molecules using cyclodextrin-based nanostructured materials.

10:45 AM NN6.8
NANOPARTICLES AND POLYMERIC VESICLES FROM NEW POLY-L-LYSINE BASED AMPHIPHILES.
Ijeoma F. Uchegbu, Wei Wang, University of Strathclyde, Dept of Pharmaceutical Sciences, Glasgow, UNITED KINGDOM; Laurence Tetley, University of Glasgow, Institute of Biomedical and Life Sciences, Glasgow, UNITED KINGDOM.

Nanoparticles and polymeric vesicles for drug delivery and other industrial applications have been prepared by simply sonicating specially designed poly-L-lysine graft copolymer amphiphiles in aqueous media. Amphiphiles which have a poly-L-lysine backbone and varied levels of both hydrophilic methoxypolyethylene glycol (Mw ~5,000) and hydrophobic palmitoyl pendant groups, were prepared from 2 different molecular weight poly-L-lysine hydrobromide samples (~4,000 and ~20,000 respectively). These amphiphilic polymers (PLPs) were characterised using light scattering, 1H NMR and an assay for the level of free amino groups. Steric factors appear to limit the final level of lysine group modification that can be achieved and even an excess amount of grafting reactants still resulted in the production of polymers in which 22 - 26 mole% of the lysine terminal amino groups remain unsubstituted. Probe sonication of an aqueous dispersion of PLP samples resulted in the production of stable nanoparticles (80 -170nm in diameter) as imaged by electron microscopy. Nanoparticles were able to encapsulate the hydrophilic fluorophore FITC-dextran and encapsulation increased as the level of unreacted lysine terminal amino groups in PLP increased, indicating the presence of hydrophilic microdomains. Polymeric unilamellar vesicles (220 - 570nm in diameter) imaged by electron microscopy were produced by probe sonication of PLP, cholesterol. Vesicle formation was possible over a narrow spectrum of polymer architecture and was favoured by a low molecular weight and a low level of palmitoyl substitution. A vesicle formation index has thus been derived. The size of both the nanoparticles and the vesicles was directly influenced by the molecular weight of PLP. PLPs of molecular weight 32,000 - 48,000 and 89,000 - 140,000 resulted in nanoparticles of 85 - 114 nm and 125 - 167 nm in diameter respectively and PLP of molecular weight 25,000 and 89,000 gave rise to polymeric vesicles of 252 nm and 570 nm in diameter respectively.

11:00 AM NN6.9
ENGINEERING LARGE MONODISPERSE UNILAMELLAR VESICLES WITH HIGH ENCAPSULATION YIELD.
Sophie Pautot, David A. Weitz, Harvard University, Dept. of Physics and DEAS, Cambridge, MA.

A vesicle is a membrane closed on itself to form a bag that delimits two volumes of the same fluid: an outer volume, and an inner volume. The advantage of this structure is that the inner fluid can contain soluble components: ions, vitamins, proteins, and polymer like DNA which are then separated and protected from the outer fluid. The membrane is composed of a lipid bilayer and these structures are called liposomes. They have been extensively used for transdermal and injectable delivery of bioactive molecules. We have developed a novel emulsion technique which allows one to produce controlled monodisperse vesicles not only with lipids but also with food surfactants and polymers. By using an inverted emulsion we define the size distribution from 200nm up to 2μm, control the lipid composition of each layer of the bilayer independently and achieve an encapsulation yield close to 100%. This technique is based on a variation of an inverted emulsion technique that disperses small droplets of aqueous solution in an organic phase. We first prepare an inverted emulsion with the aqueous solution we want to encapsulate. This is emulsified in an organic continuous phase using surfactant like molecules to stabilize the emulsion. At this step, using standard emulsion techniques we can control the size of the emulsion and the encapsulation yield. To complete the bilayer we let the inverted emulsion droplets sediment across a surfactant layer at the oil/water interface. The droplets take with them the second lipid layer to complete the lipid bilayer.

11:15 AM NN6.10
PHASE COMPOSITION DETERMINATION IN PLLA-PLGA DOUBLE WALLED MICROSPHERES.
Nausheen Rahman, Edith Mathiowitz, Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI.

We have demonstrated the fabrication of double walled microspheres from a mixture of two polymers poly(L-lactide) [PLLA] and poly(L-lactide-co-glycolide) 50:50 [PLGA 50:50] resulting in a core of polymer A rich phase and a shell of polymer B rich phase. It is important to obtain a composition profile of these spheres so that we can evaluate the arrangement of the two phases and eventually manipulate these phases to design improved double walled systems. So far it has been a difficult task due to inherent problems with determining the composition of the spheres but with the current technique described in this paper some of the problems have been overcome. In addition PLLA and PLGA are chemically so similar that it is not always easy to identify and separate the different peaks in characterization techniques such as the fourier transform infrared

spectroscopy. Therefore we had to resort to the differential scanning calorimetry to quantify the amounts of PLLA and PLGA 50:50 in our double walled spheres. The composition profiles of the microspheres were compared to compositions of phase separated polymer mixtures at different concentrations in an attempt to understand the thermodynamics behind the fabrication of double walled spheres from such polymer mixtures. In addition we wanted to determine the effects of drug incorporation on both the compositions and the arrangements of the different phases in the spheres. If phase arrangement and drug localization are indeed related in double walled spheres, we can try manipulating the phase arrangements to control the layer in which the drug is encapsulated. This would have a tremendous impact on the drug release profile using double walled spheres.

11:30 AM NN6.11
Abstract Withdrawn.

11:45 AM NN6.12
NEW POLYMER SYSTEMS SYSTEMS WITH CONTROLLED RELEASE ACTION. Apostolos K. Rizos[1], John Alifragis[1], Aristidis M. Tsatsakis[2], Manolis Tzatzarakis[2], Michail Shtilman[3]. [1]University of Crete, Department of Chemistry, Heraklion, GREECE. [2]University of Crete, The School of Health Science, Heraklion, GREECE. [3]Mendeleev University of Chemical Technology, Moscow, RUSSIA.

The last few decades have witnessed concerted efforts to enhance the effectiveness of drugs used in therapeutic, diagnostic and preventive medicine. Many of the problems associated with conventional drug therapy may be circumvented by the use of delivery systems which in a variety of ways will optimize drug action. The concept of targeted drug delivery was first aired early this century and entails the use of carrier systems to deliver drugs to where they are needed or facilitate their release there. In the present work we employed dynamic light scattering to obtain a comprehensive dynamical measure of a series of polymeric derivatives that are able to release a bioactive compound at a certain rate. Our new biocompatible polymeric systems inhibit fungal growth and mycotoxin formation.