Nanoscience and Nanotechnology:
Shaping Biomedical Research

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These are the abstracts of posters and exhibits that were presented at the NIH symposium, *Nanoscience and Nanotechnology: Shaping Biomedical Research*. The poster abstracts are organized into "sessions" by subject, which begin on the page numbers listed:

Exhibits by commercial vendors were solicited on the basis of exhibitors having a product at the nanometer scale that was at or near commercialization and with direct relevance to biology or biomedicine. The intent of including exhibits by commercial vendors was to stimulate discussion among the symposium participants and provide a preview of some of the exciting potential for solutions to medical and biological research problems that will be enabled through further research and development in the areas of nanoscience and nanotechnology. Exhibits by national nanofabrication resources also were included.

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Devices for Early Disease Detection and Single-Cell and Molecule Measurements

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1 Sensing, Planning, and Control for Nanomanipulation Probes

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This research effort seeks to develop devices capable of nanomanipulation in biomedical applications, eventually, applying the new planning and control scheme to transgenic (*in vivo*) manipulations. The new planning and control method for nano manipulation will also be applied to the study of the complex regulatory relationships among transcription factors critical for early *Drosophila* embryogenesis.

The methods emanate from fundamental understanding of the physics related to nano manipulation and the development of integrated sensors and controls. The interdisciplinary methods used stem from engineering, physics, and biology. Kinematics and dynamics modeling in nano manipulation involves the development of models for nanoforces, using experimental engineering testing and verification. The models provide foundations for design, sensing, processing, planning, and control. This requires human interface such as haptic display, which is essential information for nano manipulation. Thus, the development of a technology to map the haptic information from the nanoworld to the macroworld for human-device interface is required. Planning and control of nano manipulation includes developing hardware and software systems for nano manipulation. The effort focuses on two aspects: developing a sensor-referenced planning and control to facilitate an autonomous operation and developing a human/machine interface to achieve a human and autonomous cooperative controller.

The results to be presented articulate our manipulation and control framework using imaging and motion haptics to repeatedly and accurately control nanomanipulating probes in 3Dimensional environments.

2 Transmission Electron Microscopy Imaging of Gold Dendrimer Nanocomposites

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Poly-amidoamine (PAMAM) dendrimers are composed of b-alanine subunits and have great potential in biomedical and materials science applications, due to their predetermined size, shape and surface functionalities. These spherical, artificial proteins may be functionalized and used as nanoscopic building blocks. However, PAMAM dendrimers are aliphatic and have protein-like structures; therefore, they are extremely difficult to observe directly in cells or in tissue. Commonly, fluorescent markers or surface complexes of metal ions, such as Gd3+, are used to overcome this obstacle. Nonetheless, conjugation of markers to the surface of the macromolecule may dramatically change not only the solubility, but other surface-related properties as well. This problem can be solved by using dendrimer nanocomposites (DNCs) that are easy to observe by transmission electron microscopy (TEM). In this work, gold (0) PAMAM dendrimer nanocomposites, {Au(0)n-PAMAM} have been selected to demonstrate this nanoparticle-based concept of imaging. Gold nanocomposites offer the high-electron density of the guest atoms, while their interactions with the environment are determined by the surface of the host dendrimer molecule. As a consequence, an inorganic particle can be manipulated as if it were organic, and monodisperse nanoparticles with various surfaces may be created. {Au(0)n-PAMAM} gold dendrimer nanocomposites with a well-defined size (5 nm) have been synthesized with cationic, anionic, or neutral surfaces and have been tested for use in TEM imaging both *in vitro* and *in vivo*. The results demonstrate the advantages of gold nanocomposites that are observable in TEM (with or without additional staining): They readily penetrate into cells, and their interaction with the biological objects is influenced by the properties of the template dendrimers.

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3 Microscale Detection of Biological Species in Microfluidic Chips

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The development of techniques to process and detect biological species using microfabricated systems will accelerate the practical applications of chip-based assays and ultimately will permit the completion of all of the operations on a chip, using minute sample volumes. In the first project, the design and fabrication of microfluidic chips are being studied for affinity-based detection of biological species. The chip consists of a series of cavities or wells, which are anisotropically etched in silicon wafers, connected by etched channels, and sealed with a glass cover. Platinum electrodes are defined at the bottom of the cavities, the surfaces of which are oxidized, and these serve as the primary sensing and reaction elements, when coated with receptors, antibodies, enzymes, etc. A spin-on glass is used as an intermediate layer to bond the glass cover to the oxide-covered chip. The goal is to study all-electronic detection methods, such as impedance changes, upon affinity binding of the target molecules and/or cells to the sensing species immobilized on the surface of the electrode. The protein Avidin has been adsorbed on the chip surface but does not loose its conformation, and can still bind to Biotin (a vitamin) upon subsequent reactions. The adsorption and ensuing binding have been confirmed using optical fluorescence microscopy. The Avidin-Biotin system can be exploited as a universal attachment scheme on the chip surface for any species that can be biotinylated. At the same time, these devices are being used to study voltage-assisted adsorption of the sensing species on the electrodes, which will make the immobilization process faster and more selective to the surface.

Grants: NSF
USDA

4 A Novel Bio-Atomic Force Microscope Cantilever

Arthur Beyder and Frederick Sachs, Ph.D., *Department of Physiology and Biophysics, State University of New York at Buffalo*

Atomic Force Microscope (AFM) cantilevers are designed to be used in air, a low viscosity medium. However, in biology experiments, the substrates are hydrated, so the experiments are performed in liquids in which viscous drag profoundly affects the cantilever's performance. A bio-AFM cantilever needs to be soft (<0.1 N/m), with a minimal moving area (to reduce viscous drag), while at the same time it must maintain a resonant frequency to minimize scan times. We have fabricated a new design bio-AFM cantilever, with stiffness <0.01 N/m and moving areas that are 12-15 times smaller than the commercially available cantilevers, which permits a great reduction of viscous drag. The smaller length of these cantilevers increases the sensitivity inversely with the length. This allows the cantilever to be made stiffer for the same transfer function of force to angle. In turn, this increases the bandwidth.

The cantilevers are fabricated via a 5-layer microfabrication process. Using a backside KOH etch, thin membranes (~10 um) are made. Three masking thin films are deposited onto the topside to pattern the cantilevers into the silicon membranes. A LPCVD Silicon nitride film of 100 nm is a masking layer from the topside and a hinge layer from the backside. The cantilevers are patterned and released using a dry plasma etch.

We have fabricated a series of cantilevers with varying degrees of stiffness simply by varying the length, width, and thickness of the hinge layer.

The bio-AFM cantilever will prove to be an improvement over the industry standards for several reasons. First, the reduction of moving area reduces the effect of viscous drag encountered in fluids. Second, by simply varying the length of the hinge we will be able to have a series of cantilevers that vary in stiffness by a factor of 1000 on a single chip. Finally, we can have super-soft cantilevers by simply changing the thickness of the silicon nitride hinge, without having to change the overall design of the cantilever.

5 Rapid Solution Switch for Electrophysiology Experiments

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The patch-clamp technique allows the study of receptors on the cell membranes with time resolutions approaching microseconds. However, for experiments that involve concentration changes at a membrane patch, the speed of the solution exchange is the limiting factor. The most effective technique for fast solution switch involves the movement of the patch across two closely spaced streams under laminar flow conditions. We have used Si nanofabrication technology to make a dual-flow perfusion chip with the partition width at the outflow of 1 µm. The device should allow us to change concentration at a patch in less than 10 µm.

In order to make enclosed fluid channels, our device was fabricated using two chips with complementary general shapes and channel geometry on the top and bottom. The bottom chip houses multiple input paths with small (20X30 µm) channels at the exit port. These channels join near the outlet to form the test channel. Another channel runs the length of the chip as a control channel. The tip of the wafer has two outlets (one from the test solution manifold and the other from the control), separated by a 1 µm partition.

We have fabricated the top and bottom chips using photolithography and plasma etching techniques. Surprisingly, the main problem has been the bonding of the top to the bottom without leaks or clogging the sharp cornered flow paths. Tests are in progress.

Finite element simulations suggest that we should be able to achieve a 5-95 percent concentration jump in less than 10 µsec, a 20- to 30-fold improvement over the traditional theta-glass setup. Moreover, the small volume of the device minimizes the loss of potentially expensive/ rare reagents. The flow volume at 30-40 nl/min/channel is more than 100,000 times smaller than the traditional switchers.

6 Endothelial Cell Attachment to Micro-Patterned Octadecytrichlorosilane Self-Assembled Monolayers

Shalanda R. Webb, Donna R. Weinbrenner, and Thomas Boland, Ph.D., Department of Bioengineering, Clemson University, Clemson, South Carolina

Cell response to patterned materials was examined by employing highly organized self-assembled monolayers (SAMs) of octadecytrichlorosilane (OTS) on silicon wafers. SAMs are candidates for ultra-high-resolution lithography because they form ultra-thin layers that are sensitive to modification by electron-beam etching through a mask. Furthermore, pure OTS monolayers are poor substrates for cell growth, most likely because of the denaturing of serum proteins near the surfaces. The goal of this research is to use lithographical techniques in designing patterns to which endothelial cells may attach.

Pure OTS surfaces were prepared by self-assembly from solution. In order to induce micro patterns, the surfaces were exposed to ozone through a mask. The resulting patterns were examined by atomic force microscopy (AFM), ellipsometry and x-ray photoelectron spectroscopy (XPS). Bovine endothelial cells were cultured in DMEM in the presence of serum. Standard biochemical tests were used to determine cytotoxicity of the surfaces; SEM and light microscopy were used to visualize the attached cells.

After etching, the thickness of the OTS layer was reduced from 22 Å to 10Å, as judged by ellipsometry and AFM. High-resolution XPS C1s scans revealed a shoulder in the hydrocarbon peak of the etched samples, indicating the carboxylated species. SEM revealed few cells attached to the pure OTS surface. However, on patterned OTS the cell density increased in regions of the pattern to a near confluent layer. The cell spreading and attachment on the micropatterned surfaces suggests that the cells may be able to attach more firmly to the extracellular proteins on the patterned surfaces.

7 Synthesis and Relaxometric Investigations of G=7 and G=9 Starburst Dendrimer-DOTA-M Chelates, Where M = Gd(III), Mn(II), and Fe(III)

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Starburst poly-amidoamine (PAMAM) dendrimers have uniform surface chemistry and minimal molecular weight distribution and shape variation. The uniform-surface chemistry allows for reproducible chemical derivatization. We have covalently attached derivatized macrocyclic chelates to the surface of the generation-7 and -9 dendrimers. The appropriate metal ions useful as magnetic resonance imaging (MRI) agents—such as gadolinium, manganese, and iron—were coordinated to the dendrimer-based macrocyclic chelates. We have been able to synthesize the largest number of metal ions per macromolecule (i.e., dendrimer) that has ever been reported. The relaxometric investigation, which is a measure of the relation rates per mM concentration of metal ion as a function of magnetic field strength, shows that at the clinically relevant field strength of 1.5 T, the dendrimer-based gadolinium chelates are approximately 4 times more efficient than the clinically used monomeric gadolinium chelates. We have explored the potential of the new dendrimer-based gadolinium chelates for use in MR angiography. Delineated vascular visualization in the rat was observed for at least one hour. These results demonstrate the potential of dendrimer-based chelates for use as MR-contrast agents.

8 Magnetodendrimers as a New Class of Cellular Contrast Agents

J.W.M. Bulte, Ph.D., T. Douglas, Ph.D., E. Strable, B.M. Moskowitz, Ph.D., and J.A. Frank, M.D., Laboratory of Diagnostic Radiology Research, Clinical Center, National Institutes of Health, Bethesda, Maryland

Objective: We have developed magnetodendrimers as a potential new class of MR contrast agents, and explored these agents for the preparation of magnetically labeled cells (MagTag).

Methods: Carboxyl-terminated dendrimers were incubated with 100 Fe(II) atoms per molecule. Following oxidation at elevated pH and further purification, MD-100 was analyzed by transmission electron microscopy, X-ray- and electron diffraction, SQUID magnetometry, and variable field T1-T2 relaxometry. Human mesenchymal stem cells, small cell lung carcinoma, cervix carcinoma, and rat glial cells were co-cultured with MD-100 at 10-25 mg Fe/ml for 48h.

Results: MD-100 consisted of an oligocrystalline structure of 7-8 nm crystals, with a highly ordered inverse spinel maghemite-like structure. MD-100 exhibited a saturation magnetization of 94 emu g/Fe with no hysteresis. The T1 and T2 relaxivities were 11 and 200 $mM^{-1}s^{-1}$ at 37 °C and 1.5 tesla, respectively. Prussian Blue staining of MagTagged cells revealed a cytoplasm that was completely filled with iron-containing vesicles. As compared to nonlabeled, matched control samples, the 1/T2 of MagTagged cell suspensions showed a dramatic increase from approximately 1.0 to 50-150 s^{-1}. MagTagged cells could also be easily separated within 10 seconds, using a 1200 Gauss/cm permanent NdFeB magnet, with full resuspension, allowing magnetic manipulation.

Conclusions: Magnetodendrimers represent a new class of superparamagnetic agents that have desirable (magnetic) relaxation properties and that show a high, nonspecific affinity for cellular membranes. Magnetodendrimers can label cells regardless of their origin or animal species; this opens up the possibility of MR tracking of a wide variety of MagTagged-cell transplants.

9 Magnetic Resonance Tracking of Cell Migration and Myelination Following Transplantation of MagTagged Glial Cells

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Objective: Transplanted glial cells and their precursor cells can (re)myelinate axons, raising the possibility of therapeutic intervention in myelin disorders. We have tagged grafted cells with a magnetic label in order to track their migratory capacity and induced myelination non-invasively using high resolution magnetic resonance (MR) imaging.

Methods: Iron oxide nanoparticles were shuttled into cells using a covalently linked anti-transferrin antibody construct. Approximately $5x10^4$ MagTagged viable (n=5), MagTagged paraformaldehyde-killed (control, n=2), and untagged (control, n=5) cells were grafted in the spinal cord of neonatal myelin-deficient (md) rats. At either 10 (n=4) or 14 (n=8) days following transplantation, spinal cords were removed and imaged at 78 micron isotropic resolution

Results: The 3D images showed extensive migration (up to 8.4 mm) of grafted cells, in particular in the area of the dorsal column. No spreading of contrast could be observed in the control-graft MR images. Histopathological correlation showed an excellent agreement between the observed MR contrast and areas of Prussian Blue- and myelin-positive staining.

Conclusions: MagTagged glial cells fully retain their migration- and myelinating capacity *in vivo*. MR tracking of neurotransplanted cells appears feasible and may be used to accurately map the achieved extent of (re)myelination.

10 Silicon-Based Microcavity Nanosensors for the Detection of Biomacromolecules

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The large surface area of porous silicon provides numerous sites to which many potential species to attach, making it an ideal host for sensing applications. The average pore size can easily be adjusted to accommodate either small or large molecular species. When porous silicon is fabricated into a structure consisting of two high-reflectivity multilayer mirrors separated by an active layer, a microcavity is formed. Multiple narrow and visible luminescence peaks are observed with a full width at half the maximum value of 3 nm. These multiple peak microcavity resonators are highly sensitive structures. Any slight change in the effective optical thickness induces a shift in the interference peaks; therefore, the reflectivity spectrum is a sensitive indicator of the attachment of biological objects. We demonstrate the usefulness of this microcavity resonator structure as a DNA biosensor. A probing strand of DNA is initially immobilized in the porous silicon matrix, and then subsequently exposed to its sensing complementary DNA strand. Red-shifts in the luminescence spectra are observed and detected for DNA concentrations in the micromolar to femtomolar regime. The spectral shifts confirm successful recognition and binding of DNA molecules within the porous matrix. The detection scheme also has been extended to include the detection of viral DNA. Porous silicon microcavity resonators have shown great potential for biosensing as the sharp spectral features of the luminescence can reveal very small changes in refractive index. A detailed study on the selectivity and sensitivity issues of porous silicon microcavity biosensors will be presented. This work will someday lead to the development of a "smart bandage," through which the detection of bacteria or viruses can be diagnosed and an antibiotic treatment can be recommended.

Grant: W.M. Keck Foundation

11 Novel Biosensors Based on Nanosized Conjugated Polymer: Detection of Hepatitis C Virus and Broken DNA Strands

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An entirely new class of highly sensitive, selective, robust, and economical biosensors for rapid detection of Hepatitis C virus and broken DNA strands has been developed. These are based on a recent scientific breakthrough in which the photophysical properties (e.g., fluorescence) of nanosized conjugated polyelectrolytes can be greatly altered by certain types of electron-acceptor molecules with a quenching sensitivity 10-million-fold higher than that for conventional small molecule-based materials. Coupling the reversal of quenched polymer fluorescence with a bio-recognition event, we constructed a "turn on" biosensor, which is capable of identifying toxins, viruses, and broken DNA strands at nano to pico molar concentrations. Herein, diads that contain the quencher molecules linked to bio-ligands such as a recombinant antibody (single-chain Fv) for Hepatitis C core protein or a DNA-binding domain of poly(ADP-ribose) polymerase have been synthesized. In the absence of virus or broken DNA strand, these diads efficiently quenched the polymer fluorescence. Yet, by adding small amounts of the bioanalytes, a dramatic increase in fluorescence was observed due to the antibody-antigen interaction or complex formation between the DNA-binding protein and broken DNA strand. It is anticipated that this novel sensor array could replace currently used ELISA and related assays in the clinic for identification of Hepatitis C virus in serum. It will also provide a new tool to study DNA damage and repair mechanisms inside cells.

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12 Detection of DNA-Hybridization by Nanobead Labeling: New DNA-Chip Readout Schemes with the Potential of Single-Molecule Detection

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Highly parallel detection of DNA hybridization using microarrays shows tremendous promise for medical, pharmaceutical, forensic, and other applications. For detection of specific molecular binding, the arrays are incubated with a solution containing binding partners of the immobilized molecules. Specific binding is visualized by use of tagged molecules. Besides radioactive labeling with its inherent safety problems, fluorescence labels are broadly used in standard applications of microarrays. Although hampered by the need for sophisticated fluorescence microscopes/scanners as well as strongly environment-dependent quantum yields, no other scheme for readout could supersede fluorescence detection for standard use.

We present new detection schemes based upon nanobead (colloidal gold) labeling of the DNA. The binding of individual nanobeads can be visualized by atomic force microscopy (AFM), demonstrated by a quantitative study of the influence of nanobead concentration in solution on the density of surface-bound nanobeads. However, AFM detection is serial and therefore slow.

A simple optical detection without fluorescence is presented, which is sufficient to detect the nanobead-labeled DNA in a highly parallel manner on a chip. A high signal with a significant specificity of hybridization could be demonstrated either in reflection or in absorption mode.

The potential of nanobead labeling for electrical detection is discussed and illustrated by preliminary experiments. The presence of nonspecifically deposited nanobeads in an electrode gap could be detected by electrical measurements. Immobilization of the oligonucleotides on chips based on silanization, as well as ultramicroscopical methods (AFM, TEM) adapted for chip characterization and first designs of nanoelectrodes, will be presented.

13 Nanostructured Surfaces for Optical Amplification of Ligand-Receptor Binding Using Liquid Crystals

Vinay K. Gupta, Justin J. Skaife, Timothy B. Dubrovsky, and Nicholas L. Abbott, University of Wisconsin-Madison

Liquid crystals (LCs) are used to amplify and transduce receptor-mediated binding of proteins at surfaces into optical outputs. Nanostructured surfaces are designed so that protein molecules, upon binding to ligands hosted on these surfaces, trigger changes in the orientations of 1-20 micrometer-thick films of supported LCs. This corresponds to a reorientation of ~10^5-10^6 mesogens (molecules that form the liquid crystal) per protein. Binding-induced changes in the intensity of light transmitted through the LC are easily seen with the naked eye and can be further amplified by using surfaces designed so that protein-ligand recognition causes twisted LCs to untwist. This approach to detection of ligand-receptor binding does not require labeling of the analyte, does not require the use of electroanalytical apparatus, provides a spatial resolution of micrometers and is sufficiently simple that it may prove useful in biochemical assays and imaging of spatially resolved chemical libraries.

14 Reconstructing Interaction Potentials from Perturbations to the Thermally Driven Motion of an Atomic Force Microscope Cantilever

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The Atomic Force Microscope (AFM) is a powerful tool for measuring forces between macromolecules and macromolecular assemblies in biological systems. The most widely used approach for these measurements is to record the DC deflection of the AFM cantilever as a function of sample position (a force curve). Here we describe an alternative approach to force measurement based on perturbations to the thermally driven motion of the AFM cantilever, resulting from interaction potentials between the tip and a sample. From the perturbations in the thermal oscillations, we calculate the interaction potential and reconstruct the interaction force as a function of tip-sample distance. As a test case, we apply this analysis to an electrostatic double well potential, isolate the tip-sample interaction potential by removing the cantilever potential from the total potential, reconstruct the DC force curve from the interaction potential, and present 3D representations of the interaction potential. We also show this approach can be expanded in two ways. First, driving the cantilever with a forcing function with a white noise power spectrum simulates higher cantilever temperatures, and the cantilever can explore potentials deeper than several kT. Second, the approach is shown to generalize to include forcing functions of any type. In other words, the driving function can be modified to force the cantilever to spend more time in particular regions of the potential than it may thermally, thereby improving the sampling in those regions. This approach to force measurements can provide detailed descriptions of the interaction potentials between single molecules.

15 Driven DNA Transport into an Asymmetric Nanometer-Scale Pore

Sarah E. Henrickson, Martin Misakian, Baldwin Robertson, and John J. Kasianowicz, Biotechnology and Electricity Divisions, National Institute of Standards and Technology, Gaithersburg, Maryland

To understand the mechanism by which individual DNA molecules enter nanometer-scale pores, we studied the concentration and voltage dependence of polynucleotide-induced current blockades of a single alpha-hemolysin ion channel. We show the blockade frequency is proportional to the polymer concentration and increases exponentially with the applied potential and that DNA enters more readily from one side of the pore. We also measure directly the value of the electrical potential that confines a modified version of the polymer inside the pore against random diffusion and repulsive forces.

16 Rapid Multianalyte Detection with a Nanopore

John J. Kasianowicz, Sarah E. Henrickson, Howard H. Weetall, and Baldwin Robertson, Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland

Rapid and simultaneous measurement of many analytes represents the next frontier in sensing. New measurement techniques will make feasible or reduce the cost of analyzing blood, detecting pathogens and toxins in drinking water, and chemical and biological warfare agents. The most promising new methods use ion channels, which perform transport and defense functions in cells and organelles. Some channels act as sensors by converting the concentration of an analyte into a change in the pore's conductance. Recently, several groups suggested that channels placed in artificial membranes might prove useful for detecting analytes. Unfortunately, only highly specific classes of analytes (e.g., neurotransmitters, anesthetics, protons, or deuterium ions) can be detected by a change in native channel conductance. Some steps towards adapting channels for analyte detection have been taken by placing recognition sites inside the pore, adjacent to the pore's mouth, or well outside the pore. We demonstrate here that a wider variety of analytes can be detected simultaneously by a simpler system. Instead of attaching the recognition elements to a channel, we covalently link them to polymers that thread completely through a pore.

17 Bioanalytical Applications of Single Molecule Detection

Richard A. Keller, James H. Jett, and Babetta L. Marrone, Los Alamos National Laboratory, Los Alamos, New Mexico

We have developed the capability to detect and identify single fluorescent molecules as they flow through a focused laser beam. Sensitive detection is an important aspect of nanotechnology. A modern trend in analytical and material sciences is to make devices smaller and faster with the ultimate goal of creating devices at the molecular level. At this level, sensitive detection is a major issue.

Single molecule analyses unmask the intrinsic properties of individual molecules hidden in ensemble averages. Additionally, information important to characterizing a system—such as heterogeneities, properties of trace constituents, and differences in reaction pathways—becomes visible. Often, trace constituents or minor reaction pathways are the most important in determining the properties of a system.

We will describe our approach to single-molecule detection in flowing solutions and the application to bioassays that would be difficult or impossible in bulk solutions. Particular attention will be paid to DNA fragment sizing.

18 Light-Powered Microtransponders for Biomedical Applications

Wlodek Mandecki, Natan Kogan, and Zhuying Wang, PharmaSeq, Inc., Monmouth Junction, New Jersey

A fully functional microtransponder was developed for a novel bioassay system that will be capable of accurately detecting and differentiating a large number of molecules. The microtransponder is one of the smallest remotely powered electronic devices made to date and is capable of processing and transmitting a radio-frequency signal. Applications include nucleic acid diagnostics, drug discovery, immunoassay, and others. Microtransponders are cube-shaped, miniature radio-frequency transmitters, only hundreds of micrometers on each side, manufactured by photolithography techniques with the feature size well below 1 micron. The microtransponder identifies itself by transmitting a radio signal when illuminated by a beam of laser light. The signal transmitted by the microchip contains 50 bits of data, sufficient to provide more than 10^{15} different identification numbers or sequences.

In DNA diagnostics, the presence of complementary DNA sequences in a biological specimen is determined by reacting fluorophore-labeled-specimen nucleic acid with transponders, each derivatized with a different oligonucleotide probe. A scanner then detects and measures the fluorescent signal generated by the labeled specimen nucleic acid hybridized to the probe on the transponder. At the same time, the detector (scanner) identifies the nucleic-acid sequence involved by means of laser activation of the transponder's memory. The sequence is then transmitted to the scanner by radio frequency. Presently, PharmaSeq is working to optimize the hardware and assay methodologies.

The recent results pave a way for providing power for many other miniature electronic devices developed using nanotechnology techniques and for radio communication with wireless MEMS.

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NIH SBIR Phase II: 2R44HGO1532-02

19 Detection of Unusual DNA Structures with Nanoparticles

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The generic global structure of the DNA double helix is well known, but at the base-pair level, a multitude of deviations from the "ideal" exist. These deviations can result in sequence-directed curvature of DNA over a few base pairs. The most commonly studied sequences that are intrinsically bent (according to gel electrophoresis mobility studies) are A-tracts (5'-AnT-3', n = 5,6) and 5'-GGCC-3'. The GGCC sequence shows a distinct kink in its crystal structure as well. We have been able to use luminescent mineral nanoparticles of CdS in optical assays to detect these curved DNAs. The nanoparticles, originally developed by the materials science community as "quantum dots," are ~20-100 Å in diameter, similar to proteins, and their photoluminescence is sensitive to the presence and nature of adsorbates. In our assays, we use ~40 Å dots that are surface modified with either cations or hydrogen-bonding groups to bind to oligonucleotides containing sequences that lead to curvature. We find that the kinked DNA sequence binds preferentially to the generic curved surface of the nanoparticles. More recently, we have investigated higher-order structures of nucleic acids that are correlated with human disease: triplet repeats of the single-stranded oligonucleotides 5'-(CCG)n-3' and 5'-(CGG)n-3'. These sequences fold into structures that have not been determined, but give characteristic spectra in circular dichroism spectroscopy. Under salt conditions where 5'-(CCG)7-3' and 5'-(CGG)7-3' fold into these higher-order structures, our nanoparticles bind them well but do not bind to normal double-helical

DNA. This result may form the basis for future assays of higher-order DNA structures.

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20 Nanobar Codes: A Powerful New Approach to Multiplexing

Michael J. Natan, Ph.D., SurroMed, Inc., Palo Alto, California

We have developed a novel approach to labeling molecules that involves attachment of a nanometer-scale bar code and have in parallel developed hardware and software for reading those codes. In contrast to organic dyes and inorganic quantum dots, the readout does not involve fluorescence, but rather is based on a more simple and durable optical method. What makes this a potentially revolutionary advance in bionanotechnology are the facts that: (1) thousands of distinguishable nanobar codes can be prepared; (2) the sizes of the bar codes are small enough to permit very high-level multiplexing in extremely small volumes (e.g., in the interior of a cell); and (3) the bar-code reader potentially can be adapted to allow point-of-care diagnosis. This poster will describe the physical principles behind this new technology and will present recent applications.

21 Biosensors Based on Specific Binding of Magnetic Microbeads to Functionalized Surfaces

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We are developing antibody and DNA array biosensors based on the specific binding of magnetic microbeads to receptor-patterned surfaces. A critical component of each assay is the application of a controlled force to remove any beads that are not bound by specific ligand-receptor interactions. The remaining beads are then counted to determine the concentration of each of the target ligands. In the Force Differentiation Assay (FDA), magnetic force is used to remove the nonspecifically bound beads, and those remaining are detected optically. Fluidic forces are used in the Bead ARray Counter (BARC) sensor, and the specifically bound beads are detected by an array of microfabricated magnetic field sensors. A prototype FDA system using immobilized antibodies as the receptors has been successfully field tested and has demonstrated high sensitivity and specificity for proteins, viruses, and bacteria. Further development is underway to reduce the assay time, increase the sensitivity, and achieve more complete automation. In the prototype BARC system, single-stranded DNA probes are arrayed on the sensor chip above the sensor elements. When complementary DNA is present in the sample, it hybridizes with the immobilized probes; labeled microbeads are then introduced that specifically bind to this captured DNA. Because both sensor systems can detect a single microbead, in theory, each could detect a single pathogen or strand of DNA, giving them great potential for a wide range of clinical and pharmaceutical assays. The required components of each system, including fluidics, surface chemistry, and surface patterning, will be discussed.

22 Active Sensor Arrays for Biomarker Detection (*In Vivo* and *In Vitro*)

Martin Peckerar, Ph.D.[1] and Leonard Tender, Ph.D.[2]

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This poster describes the use of active arrays of solid-state sensors in the detection of biomarkers of importance in allergic response and in oncogenesis. Individual sensors respond to changes in local environmental capacitance, and thus do not require molecular charge for applicability.

Analytes are selectively ligated to sensor surfaces using self-assembled monomers. Large arrays (thousands of sensor elements) are possible in sq. mm areas. Array design considerations are aimed at eliminating the effect of nonspecific adsorption.

23 Poster Withdrawn

24 Secretion Profiles from Single Immobilized Lymphocytes Measured by an On-Line Sensor

Terry M. Phillips, Ph.D., D.Sc., Office of Research Services, National Institutes of Health, Bethesda, Maryland

Lymphocytes recovered from many biological fluids, such as cerebrospinal fluid (CSF), are usually found in extremely low numbers and yield little or no data regarding their activity. Studying the secretion of cytokines by single lymphocytes enables the investigator to determine the subtype of the cell as well as to gain some insight into its activity. A cell-capture chamber coated with immobilized ligand was devised by microforging a goblet chamber at one end of a dual-barreled micropipette. The fluid within the goblet was exchanged by passing fluid through one barrel and allowing the effluent to pass via the second barrel to a mixing chamber, where the molecules were labeled with a fluorescent dye. The labeled materials were then allowed to flow through the sensor, containing wells of immobilized anti-cytokine antibodies, for analysis by laser-induced fluorescence. Using this apparatus, it has been possible to continuously monitor cytokine secretion from T-lymphocyte subsets following stimulation with specific antigens or neuropeptides. Lymphocytes designated Th1 subtype were shown to secrete between 250-700 attograms (10^{-18}M) of interleukin (IL)-2 and gamma interferon following antigenic stimulation. Cells designated Th2 subtype did not secrete any detectable cytokines in a 72-hour period. These cells, however, did secrete 450 to 1100 attograms of IL-4 following stimulation with the neuropeptide, substance P. When coupled with a microscope-stage incubator, this system enables continuous monitoring of cell secretions over a period of 72 hours and allows examination of the role of single cell types without external influence from other cells or their by-products.

25 Fluorescent Nanocrystals for Neuroscience

Ian Tomlinson[1], Sally Schroeter[2], Laura Swafford[1], Erika Adkins[2], Scott Adams[2], Scott Ramsey[2], Louis J. DeFelice[2], Randy D. Blakely[2], and Sandra J. Rosenthal[1]

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[2]Vanderbilt University Medical Center, Nashville, Tennessee

The objective of this work is to demonstrate that fluorescent nanocrystals are a new tool for basic research in neuroscience and can potentially be used in a high-throughput fluorescence assay for drug discovery. A serotonin ligand has been used to label CdSe/ZnS core/shell nanocrystals (50Å in diameter). Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that has been linked to the regulation of critical behaviors, including sleep, appetite, and mood. The serotonin transporter (SERT) is a 12-transmembrane domain protein responsible for clearance of serotonin from extracellular spaces following release. In order to assess the potential for use of ligand-conjugated nanocrystals to target cell surface receptors, ion channels, and transporters, we have measured the ability of serotonin-labeled CdSe nanocrystals (SNACs) to block the uptake of tritiated serotonin by the human and *Drosophila* serotonin transporters (hSERT and dSERT). Estimated Ki values, the SNAC concentration at which half of the serotonin transport activity is blocked, were determined by nonlinear regression to be Ki (hSERT) = 74uM and Ki (dSERT) = 29uM. These values and our inability to detect free-serotonin indicate that SNACs selectively interact with the serotonin-recognition site of the transporter. We also have exposed the SNACs to cells containing ionotropic serotonin receptors and have measured the electrical response of the cell using a 2-microelectrode voltage clamp. We find that serotonin receptors respond to the SNACs, and we measure currents similar to the free serotonin response. We have used fluorescent SNACs to image serotonin proteins in living, hSERT transiently transfected HEK cells, and serotonergic rat neurons with fluorescence microscopy.

26 Molecular Detection with Nonbleaching Nanosized Multicolor Optical Immunolabels

David Smith, Ph.D. and David Schultz, Ph.D., Seashell Technology, LLC and University of California, San Diego

We introduce and demonstrate the use of colloidal metal Plasmon Resonant Particles (PRPs) as optical reporters in typical biological assays. PRPs are ultrabright nanosized particles that scatter light elastically and can be prepared with a scattering peak at any color in the visible spectrum. PRPs are readily observed individually with a microscope configured for dark-field microscopy, using white-light illumination of typical power.

We will illustrate the use of PRPs of different color to detect the simultaneous presence and quantity of two different analytes in a standard immunoassay. The individual target molecule detection provided by PRP labeling allows for the miniaturization of immunoassays, an important consideration for applications such as ultra-high-throughput screening, as in combinatorial drug libraries, or DNA microarrays for functional genomics studies. PRP detection labels complement other nanoparticle labels such as quantum dots and dendrimers. This technology has the potential for use in early disease detection and single-cell and molecular measurements.

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Richard Lounsbery Foundation
Seashell Technology, LLC

27 Biosensors Based on Cell Membrane Mimics

Xuedong Song, Ph.D.[1], Jeane Shane, M.S.[1], Karen M. Grace, M.E.[2], and Basil I. Swanson, Ph.D.[1]

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Fluid phospholipid bilayers either in the form of liposomes or supported on hydrophilic substrates have been shown to mimic cell-membrane surfaces. The supported lipid bilayers not only allow lateral diffusion of receptors in the surfaces but also provide a surface that minimizes nonspecific binding of macromolecules. These features make them an ideal surface platform on which to construct biosensors based on protein-receptor interactions. We have used natural and artificial receptors that are anchored to the fluid upper leaf of a bilayer membrane and attached to a reporter dye for the detection of protein toxins and pathogens that are involved in multivalent interactions. Lateral diffusion within the membrane results in binding-induced receptor aggregation, thereby triggering distance-dependent fluorescence self-quenching or energy transfer to provide reagent-free, highly sensitive, and selective detection of the multivalent proteins. The use of multiple receptors and multiple binding events gives rise to an apparent binding affinity that is much higher than the single-site binding affinity, thereby enhancing sensitivity. We have been able to detect less than 10 pM cholera toxin using pentavalent CT/GM1 interaction on lipid membrane surfaces. It is important to note that the single-site Gm1-cholera binding affinity is 0.1 micromolar, demonstrating the importance of multivalent interactions. This sensor platform has been demonstrated using fluorimetry on vesicle suspensions and flow cytometry on glass beads that have been coated with phospholipid bilayers. The optical biosensor platform also has been demonstrated, using planar optical waveguides. The platform provides a path forward for the development of a miniaturized, hand-held system that could be used in a point-of-service facility. Results for other protein systems and the prospect for adapting this approach to virtually any signature antigen will be presented.

Grant: XATX, Laboratory Directed Research and Development Funds

28 Nanoliter Protein Arrays Prepared by Microfabricated Stamps

Fan-Gang Tseng, Ph.D.[1], Haimei Huang, Ph.D.[2], Shih-Chang Lin, M.S.[1], Chang-Sheng Liu[3], Chau-Yuh Huang[1], and Ching-Chang Chieng, Ph.D.[1]

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[2]Life Science Department
[3]Power Mechanical Engineering Department, National Tsing Hua University, Hsinchu, Taiwan

Active nanoliter protein arrays play an important role in the next generation of high-throughput biomedical diagnostics. The traditional microarray methods, utilizing microneedle and robot systems to form DNA arrays, cannot be employed for active protein spotting, because the protein may be inactivated during the serial and long arraying process. A novel method using microfabricated stamps is proposed to simultaneously stamp various proteins onto a protein-receiving surface in seconds. The stamped protein arrays can either be used immediately for biomedical reaction/ diagnostic purposes or stored in a biocompatible environment for later usage.

The stamp system consists of a silicon base supporting a silicone rubber stamp with hydrophilic stamp heads. While picking up different protein samples from micro Petri dish arrays, the protein reagents adhere to the stamp heads, keeping an accurate volume in nanoliter range. A bulk and surface micromachined mold is utilized to micromold the stamps. After de-molding, a thin hydrophilic MgF_2 layer is coated on the stamp surface to convert the stamp head from hydrophobic to hydrophilic.

To test the stamping process, a PVDF (Polyvinylidene difluoride) substrate was used to bind BSA (Bovine Serum Albumin) protein delivered from the microstamp. Ponceau S was then used to stain BSA protein for protein position and concentration verification. Testing showed that the stamped protein lasted for more than 15 hours under the normal bioreaction process (i.e., water wash) and still remained active. The stamp system has the potential to replace the current microneedle array systems for many bioreagent arraying applications.

Grant: NSC 89-2215-E-007-033

29 Rotational Dynamics of Single Fluorophores

Kenneth D. Weston, Michael J. Fasolka, Jeeseong Hwang, William F. Heinz, and Lori S. Goldner, National Institute of Standards and Technology, Gaithersburg, Maryland

The extension of fluorescence spectroscopy to the single molecule (SM) regime has allowed access to a wide range of phenomena that are obscured in ensemble measurements. For example, observables—such as fluorescence intensity, spectrum, and lifetime—fluctuate in time, indicating changes in the conformation of the fluorophore or the chemical or physical environment in which it resides. Our recent work has focused on another important observable, the dipole orientation of single fluorophores. Polarization modulation is used with confocal and wide-field microscopy to measure and image the absorption dipole orientation and reorientation dynamics of individual molecules. By monitoring orientation with a time resolution of several milliseconds, we have observed both discrete and continuous dipole changes for molecules in a variety of sample types. This technique is being developed for routine use on single fluorophores embedded in biological and biomimetic samples and ultimately will improve our understanding of macromolecular motion of protein catalysts, complexes, channels, motors, and protein folding.

30 Single-Molecule Analysis in Biomedical Sciences

X. Nancy Xu, Jinsong Gao, Robert Jeffers, Hiroshi Yoneyama, and Z. Julia Wen, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Virginia

Biochemical analyses at the single-molecule level present unique opportunities to study and characterize the chemical and physical properties of individual molecules. Rare molecules, once hidden from view by an overwhelming ensemble average, can now be singled out and explored in detail. Potential applications in biomedical research that are unique to single-molecule analysis include tracking individual steps in a sequence of biological events, early detection of diseases, monitoring of the variability of biomolecular conformations, and manipulating individual biological reactions. Our research involves real-time monitoring of dynamics and interactions of single-protein molecules on/within cellular membranes, tracking of single antibiotic molecules passing through membrane transporters, and screening of single tumor marker molecules for earlier cancer detection.

In this report, we will describe real-time monitoring of dynamics and interactions of single-protein molecules for understanding of biological function (e.g., pumping machinery), design of biocompatible materials (e.g., vaccine), and invention of bio-inspired novel technologies at the molecular level. We also will discuss our recent development of single-molecule chemical microscopy for tracking biochemical reactions associated with single ligand-receptor interactions on membranes to improve understanding of the immune response pathway. Furthermore, we will present our most recent attempt to screen the mixture of tumor markers for early and high-accuracy detection of cancer. The detailed experimental configuration, updated research results, and prospective applications will be discussed.

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Biological Nanostructures

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31 Mutated Nanopores as Sensitive and Selective Detectors for Individual Ions in Solution

Daniel L. Burden, Ph.D., and John J. Kasianowicz, Ph.D., National Institute of Standards and Technology, Gaithersburg, Maryland

We are exploring the ability of genetically engineered versions of the *Staphylococcus aureus* alpha-hemolysin (aHL) ion channel, a protein that forms nanometer-sized pores in biological membranes, to serve as rationally designed sensor components for transition-metal divalent cations. Using single-channel recording, individual divalents bind to the channel and cause a momentary fluctuation in current. These fluctuations can be used to determine the type and amount of ion present in solution. We will report our selectivity and sensitivity results for a variety of mutant constructs. Through the use of scanning mutagenesis, we also show that the mutated region of the pore is lined by a beta sheet and that the sites near position 130 span a reverse turn. Both conclusions are consistent with the crystal structure of WT-aHL determined by oligomerization in detergent.

32 Optically Monitoring Single Molecules in Unsupported Lipid Bilayers with Confocal Microscopy

Daniel L. Burden, Ph.D., and John J. Kasianowicz, Ph.D., National Institute of Standards and Technology, Gaithersburg, Maryland

We are developing a new measurement technology that combines single-molecule fluorescence spectroscopy with single ion-channel electrophysiology. The technique allows the structure and function of individual membrane-incorporated molecules to be simultaneously interrogated with light and electricity in real time. As a first step toward this goal, we have coupled a highly sensitive scanning confocal fluorescence microscope to a planar lipid bilayer apparatus and have conducted optical studies of fluorescently labeled lipids at the single-molecule level.

In this presentation, we discuss the experimental apparatus for probing single molecules in unsupported planar-lipid bilayers and report on our resulting diffusion coefficient measurements. Special emphasis is given to the appearance of a significant diffusion bias at high photon flux. We hypothesize that the bias is optically induced and that it results from a resonantly enhanced optical trapping mechanism. We present both experimental and theoretical evidence that is consistent with the optical trapping and manipulation hypothesis. Lastly, we comment on the significance and potential utility of manipulating single molecules in bilayer membranes.

Grant: NAS/NRC Postdoctoral Associateship

EP1 Solvent-Free Protein Nanoparticles

Trevor P. Castor, Ph.D., Aphios Corporation, Woburn, Massachusetts

Controlled-release systems for drugs have received increased attention and application over the past decade, as they frequently provide a dosing regimen significantly superior to conventional techniques. By allowing a near-constant level of drug to be given to the patient, treatment efficacy can be greatly improved. In the formulation of controlled-release therapeutics, it is frequently desirable to disperse the material into very fine, uniform particles. With proteinaceous therapeutics, the generation of such fine particles is particularly problematic. Existing practices have difficulty achieving the desired particle-size distribution, expose the protein to denaturing conditions such as heat or air, or leave residual product contamination, which necessitates further processing.

Aphios Corporation has developed a protein comminution process that avoids the aforementioned difficulties. The patented process uses nontoxic liquefied gases, compressed gases, or supercritical and near-critical fluids that have properties intermediate to gases and liquids (referred to as *SuperFluids*Ô) to form small (1-5µm), monodisperse protein particles without first dissolving the material in a liquid solvent. The protein nanoparticles, for the most part, retain full activity and are devoid of residual processing chemicals such as solvents, salts, or surfactants. For certain enzymes, several-fold enhanced bioactivity has been observed.

Aphios Corporation's protein comminution process can benefit the pharmaceutical and biotechnology industries by yielding improved efficacy of therapeutic products with less residual contamination. The technique can be useful in obtaining a desired particle size in drug manufacture and may also prove useful in terms of controlled-release formulations such as polymeric nanospheres and aerosol delivery systems.

33 Exploring Amyloid and Pre-Amyloid Protein Assemblies by Atomic Force Microscopy

Tomas T. Ding, Ph.D., and Peter T. Lansbury Jr., Ph.D., Center for Neurologic Diseases, Brigham and Womens Hospital, Boston, Massachusetts and Department of Neurology, Harvard Medical School, Cambridge, Massachusetts

Protein aggregation seems to play a key role in many neurodegenerative ailments, the two most common being Alzheimer and Parkinson's diseases. We are investigating assembly processes of amyloid forming, disease-related proteins, such as amyloid b-protein (Ab, Alzheimer disease AD) a-synuclein (asyn, Parkinson's disease PD) and huntingtin (htn, Huntington's disease HD). Ultimately, well-ordered linear assemblies rich in b-sheet (i.e., amyloid fibrils) can be detected in solution systems of these proteins. Fibrillar structures containing Ab or asyn have been detected in or isolated from postmortem brains of clinically diagnosed AD or PD patients. It is known that both asyn and Ab form nonfibrillar oligomeric species *in vitro* at early timepoints during aggregation. In PD (and to some extent also in AD and HD), abundant data support the hypothesis that it might be these oligomeric assemblies, and not necessarily the end products (the amyloid fibrils) of the related proteins, that induce neurodegeneration. Therefore, we are focusing our research on early events of amyloid formation, and here we introduce data gathered by atomic force microscopy of systems containing aggregating nonfibrillar asyn or htn. We have, in the a-synuclein system, identified a number of distinct morphologies (such as "rings" and "chains") that might represent potentially neurotoxic species on or off the fibrillization pathway, and that could serve as possible targets for pharmacological intervention.

Grants: NS 34719
AG 08470

34 Phospholipid Monolayers Supported on Spun-Cast Polystyrene Films: Surface Characterization by Single Molecule Diffusion Measurements with Fluorescence Correlation Spectroscopy and *In Situ* Atomic Force Microscopy

John T. Elliott, Ph.D.[1], Daniel L. Burden, Ph.D.[1], John T. Woodward, Ph.D.[1], Amit Sehgal, Ph.D.[2], Jack F. Douglas, Ph.D.[2], and Anne L. Plant, Ph.D.[1]

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Supported phospholipid membranes are useful systems for a variety of biological and biotechnological applications. These membranes exhibit long-term stability and their planar topology simplifies biophysical examination of the phospholipid layer with high-resolution microscopy and other physical techniques. We are investigating the use of spun-cast polystyrene as a hydrophobic solid support for phospholipid membranes using single-molecule diffusion studies. Low-resolution fluorescent microscopy indicated that a fluorescently labeled phospholipid monolayer could be formed on the polystyrene surface by vesicle fusion techniques. Fluorescent recovery after photobleaching suggested the phospholipid layer was continuous, but diffusion studies employing real-time single-molecule fluorescence detection revealed two distinct event types: short bursts (2-5 msec) presumably due to lipids freely diffusing at ~10^{-8} cm^2/sec and rare events

(~1 sec) suggesting the presence of physical sites on the polystyrene surface that confine lipid diffusion. *In situ* atomic force microscopy revealed the presence of 20-30 nm defects on the polystyrene surface that result from prolonged exposure of the polymer film to aqueous conditions (8 hours). These defects may be responsible for the observed single molecule dynamics on the polystyrene surface. We are presently evaluating other polymer surfaces as suitable supports for phospholipid monolayers under aqueous conditions.

Grant: NRC Postdoctoral Fellowship

EP2 A Bi-Specific Ligand that Mimics the MHC Class II-T-Cell Receptor Complex and Inhibits Cellular Responses to Superantigen

Nancy M. Lehnert[1], David L. Allen[1], Beth L. Allen[1], Paolo Catasti[1], Michael Chen[2], Bruce E. Lehnert[1], and Goutam Gupta[1]

[1]Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico
[2]Texas BioGene, Inc., Richardson, Texas

Direct and external binding of *Staphylococcus* enterotoxin B (SEB), a superantigen, to the MHC class II-T cell-receptor complex bypasses the normal route of antigen processing and causes massive IL-2 release and T-cell proliferation. In this alternative mode of binding, SEB makes contact only with the a1 subunit (or DRa) of the MHC class II receptor and the variable ß subunit (or TCRVß) of the T-cell receptor. We have exploited this alternative mode of SEB binding to design a bispecific chimeric ligand, DRa-linker-TCRVß,??that acts as a receptor mimic (or decoy) and competitively prevents the interaction of SEB with its target receptors on host cells. Since the linker sequence, $(GSTAPPA)_2$, supports synergistic binding of DRa and TCRVß to SEB, nanomolar concentrations of the DRa-linker-TCRVß? construct inhibit SEB-induced IL-2 release and the proliferation of T cells. Even though this bispecific ligand binds to SEB with µM affinity, it is a better inhibitor than a high-affinity (10 pM) monoclonal antibody that binds to SEB at sites different from that of DRa and TCRVß. Therefore, design of bispecific ligands that directly target two functional sites on pathogen protein may have general applications. Currently, we are designing such bispecific ligands against the Anthrax toxin, PA, and the HIV-1 surface glycoprotein gp120.

35 Biological Nanotubules

Richard F. Hamaker, M.S., Institute for Biological Architecture, Durham, North Carolina

This poster presents a general theory of biological architecture, developed in 30 years of independent research. It suggests that carbon nanotubules are basic structures in the biological system, giving strength, flexibility, and movement necessary for feeding, growth, and reproduction.

When a carbon nanotubule is intercalated with nitrogen, oxygen, and hydrogen in a special pattern, the structure provides for energy, information, and communication functions, integrated into a single system. The theory was synthesized from established principles, starting with subatomic particles, building to the level of individual atoms then to subcellular components that are visible in electron micrographs. Many of the molecular structures identified by biochemical analysis are fragments of the carbon nanotubules.

The poster presents original drawings and pictures of scale models showing how the molecules fit together in living structures. It describes the energy system and the mechanism that moves the structures (the vital force). It then shows how the information system is organized and how it directs the flow of energy and how the information is reproduced and communicated.

The general theory provides plausible explanations for many existing (but unrelated) theories in biology. This unification and simplification at the lowest levels should stimulate advances in our understanding of the higher levels of biological organization and possible applications of inorganic nanostructures.

EP3 Unraveling Individual Nucleosomes in a Single Chromatin Fiber with Optical Tweezers Requires 20-40 picoNewton Forces

S.H. Leuba, Ph.D.[1], M.L. Bennink[2], G. Leno, Ph.D.[3], J. Zlatanova, Ph.D., Dr.Sc.[4], B.G. de Grooth, Ph.D.[2], and J. Greve, Ph.D.[2]

[1]Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
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[3]Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi
[4]Argonne National Laboratory, Argonne, Illinois

In the nucleosome ~150 bp DNA are wrapped a couple of times around an octamer of core histone proteins H3, H4, H2B and H2A, and these particles are connected by stretches of linker DNA. Polymerases must access the underlying DNA template, removing histones in a process involving force. Using optical tweezers, we have determined the force to unravel a single nucleosome.

A single-lambda DNA molecule is attached via biotinylated ends between two strepavidin-covered polystyrene beads, one held by a pipette and the other held in a force transducer optical trap. When the DNA is stretched, the force slowly rises until the contour length of B-form DNA is approached, after which it rises sharply as the DNA undergoes the B- to S- transition. A frog egg-cell extract that assembles nucleosomal arrays on the naked DNA using the endogenous extract histones is then flowed into the liquid chamber. During incubation with the cell extract, a fivefold reduction in distance between the two beads is observed in real time. Following assembly, the extract is chased out with buffer. Stretching of this single chromatin fiber produces a force versus extension curve with multiple jumps that occur principally in the range of 20 to 40 picoNewtons. These rupture steps are discrete and are of lengths of ~63 nm or its multiples.

We believe that the jumps represent unraveling of individual nucleosomes. Because an RNA polymerase can exert similar forces on naked DNA, this polymerase may be able to dislodge histones by itself.

36 A Nanoscale-Bending Motor Model of Outer Hair-Cell Electromotility

Robert M. Raphael, Ph.D.[1], Aleksander S. Popel, Ph.D.[1], and William E. Brownell, Ph.D.[2]

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[2]Bobby R. Alford Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas

Cochlear outer hair cells provide active mechanical feedback within the mammalian inner ear by generating force at acoustic frequencies in response to millivolt changes in membrane potential. We have developed a new model of this electromotile process, which proposes that the cell is organized into 40 nm motile units. The motile unit is composed of the plasma membrane, pillar proteins (which connect the membrane to the underlying cytoskeleton), and elastic cytoskeletal molecules. The plasma membrane contains molecular dipoles that rotate in response to an applied field. Dipole rotation is accompanied by a nanoscale change in membrane curvature in accord with the flexoelectric effect observed in liquid crystals. The force produced by bending is transmitted to an elastic element in parallel with the membrane identified with the spectrin molecule. The curved plasma membrane and the cytoskeletal elements comprise a nanoscale electromechanical flexion motor, which can generate forces of 100 nN/mV. The thermodynamic description of this motor is derived from an electric enthalpy representation. Nonlinearity of the mechanical and electrical response is described by the Langevin function, which specifies the fraction of dipoles oriented with the applied field. This model provides an efficient means through which cell deformation can be driven by membrane-based motors without energetically costly changes in cell- surface area. The model also explains unique observations associated with electromotility, such as reduction in membrane capacitance with pressure and voltage dependent diffusion. The outer hair cell, biology's nanoscale motor, may serve as an inspiration for the design of synthetic microelectromechanical systems.

Grants: NIH R01 DC02775, DC00354
F32 DCOO363

37 Probing the Nano-Environments of Peptides on Solid Surfaces by Advanced Secondary Ion Mass Spectrometry

T. Schenkel, Ph.D.[1], K.J. Wu, Ph.D.[2], A.V. Barnes, Ph.D.[1], M.W. Newman[1], J.W. McDonald, Ph.D.[1], and A.V. Hamza, Ph.D.[1]

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[2]Charles Evans & Associates

The interaction and bonding of peptides and proteins in the solid phase and on solid surfaces is of central importance in biotechnological research. Embedding of analyte molecules in special matrix solutions (such as 2,5 dihydroxybenzoic acid) has been shown to produce enormous increases in yields of intact molecules both for laser and singly charged ion-induced ablation. The mechanisms responsible for this enhancement are, however, not well understood. Both the binding of matrix and analyte molecules in the solid and collisional ion formation processes have been suggested to play important roles. We have investigated the effect of sodium and potassium impurities on secondary ion emission from gramicidin S by time-of-flight secondary ion mass spectrometry (TOF-SIMS) with highly charged projectiles. Highly charged ions like Xe^{44+} or Au^{69+} increase secondary ion yields by more than two orders of magnitude as compared to singly charged ions. Each highly charged projectile emits secondaries from an area of only about 10 nm^2. Analysis of coincidences among secondary ions detected following the impact of a single projectile allows for the characterization of the nano-environment of, e.g., a peptide molecule in a matrix solution. For the gramicidin S, we found that emission of Na^+ and K^+ ions was strongly correlated with emission of sodium and potassium adduct ions, $[M^+Na]^+$, $[M^+K]^+$. This correlation indicates the nestling of sodium impurities around peptide molecules. In our presentation, we will discuss the potential of coincidence analysis in TOF-SIMS for the probing of nano-environments on surfaces of biomaterials.

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38 Solvation Dynamics of Dimethyl Phosphate by First-Principles Molecular Dynamics

Eric Schwegler, Ph.D. and Giulia Galli, Ph.D., Physics Division, Lawrence Livermore National Laboratory, Livermore, California

Objective: To investigate the role of solvent interactions on the conformational flexibility of the DNA backbone.

Methods: We have used first-principle molecular dynamics simulations to examine how the conformational flexibility of the dimethyl phosphate anion $[PO_4(CH_3)_2^-]$ is altered by the presence of an aqueous environment. The molecular-dynamics simulation is based on density functional theory to compute the forces between atoms and does not rely on empirically determined parameters. Water molecules and a sodium counter-ion are explicitly included in the simulation and are described within density functional theory.

Results: In our simulation, we observe an interesting structural transformation (from gauche-gauche to gauche-trans) of dimethyl phosphate. This conformational change is preceded by an approach of the sodium counter-ion, which forms an asymmetric complex with the anionic oxygen of the phosphate group and in turn initiates the transformation. This behavior is in contrast to previous *ab initio* gas-phase investigations, which have indicated that the presence of a counter-ion has only a minimal effect on the conformational flexibility of dimethyl phosphate.

Summary: This work represents a first attempt to use first-principles molecular dynamics simulations in order to gain a better understanding of how environmental effects may influence the structure of DNA. Our results find significant differences from previous work on models of the DNA backbone and highlight the importance of treating solvent.

39 Spatial and Temporal Control of Fusogenic Vesicles Using Triggerable Lipids

David H. Thompson, Jeremy A. Boomer, Marquita M. Qualls, Junhwa Shin, and Zhi-Yi Zhang, Department of Chemistry, Purdue University, West Lafayette, Indiana

Membrane fusion processes are among the most important transient phenomena in biology, playing a central role in fertilization, synaptic transmission, cell division, intracellular trafficking, and viral infection. We have designed and synthesized a family of vinyl ether-linked phospholipids and poly(ethylene glycol) lipid conjugates that provide spatial and temporal control of lipid-mediated membrane fusion. These materials provide control over both the position and concentration gradient of fusogens within the membrane by incorporation of masked, nonfusogenic compounds that become fusogenic upon localized chemical triggering by exposure to low pH or a photooxidative environment. Cryo-TEM and vesicle leakage evidence suggests that content leakage and membrane fusion occur on two different timescales. One example of this triggerable fusogen approach uses folate-conjugated diplasmenylcholine liposomes to efficiently deliver water-soluble drugs directly to the cytoplasm of target KB cells. The targeting and triggering elements in this system work synergistically to improve the cytotoxicity of liposomal cargo, presumably via interlipidic attachments that promote membrane fusion. Cytotoxicity enhancements of 6000-fold relative to free drug were observed for cytosine arabinose in this system; efficacy also is increased by several orders of magnitude for plasmid DNA and photosensitizing drugs such as chloroaluminum phthalocyanine tetrasulfonate. Confocal microscopy evidence suggests that the primary effect of these fusogenic vehicles is to change the intracellular distribution patterns of vesicle-delivered cargo. Photocatalytic applications of these triggered vesicles for initiating enzyme cascade reactions in lithographic operations will also be presented.

40 Photochemical Micromachining of Lysozyme Crystals

Orlin D. Velev, Ph.D., Stephen R. Dziennik, Eric W. Kaler, Ph.D., and Abraham M. Lenhoff, Ph.D., Department of Chemical Engineering, University of Delaware, Newark, Delaware

Three-dimensional protein crystals are widely available, but little studied, self-assembled nanostructured materials. By virtue of the well-defined and compact structure of the protein molecules, and their efficient functionality, e.g., as enzymes or in electron transfer systems, such 3D protein lattices hold promise as biocatalysts, for biosensors, and for high- density memory storage. However, they may be of value only if it is possible to control and manipulate their sizes or shapes, which cannot be done by mechanical machining as the crystals are extremely fragile. As an alternative, we have developed ways to use light to manipulate and micromachine lysozyme crystals infused with fluorescent surfactants. Two complementary effects caused by the interaction of Uv-vis light with such crystals are reported. The first is the photochemical degradation of lysozyme in the presence of pyrene. In this case, the denatured protein subsequently dissolves and is etched off the crystal. The second is a phenomenon wherein the protein phase can be deposited, moved, or dissolved by collimated light beams. This phenomenon bears some similarity to the "light tweezers" used to manipulate colloidal particles. Both of these effects could be applied to optical micromachining of protein crystals, such as etching, drilling, cutting, patterning, or by guided deposition onto substrates. This photochemical approach may also be applied to patterning and micromachining of protein layers and hydrogels on sensors and biochips. Light-triggered bleaching or photoactivation also could open a possible way to record information into the lattice. Refs: Velev, Kaler and Lenhoff, *Adv Mater*., 11, 1345 (1999); *J. Phys. Chem. B.,* submitted.

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EP4 Evidence for a Novel Affinity Mechanism of Motor-Assisted Transport Along Microtubules

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In microtubule-translocation assays using colloidal gold particles coupled to monoclonal tubulin antibodies to mark positions along microtubules, we found that when an appropriate tubulin antibody was used, relative motion was possible between the gold particle and a microtubule gliding on molecular motors dynein or kinesin. As the microtubules moved, particles drifted to the trailing edge of the microtubule and then were released. Such motion evidently occurred by an affinity release and rebinding mechanism of the tubulin antibodies with the microtubule that did not require motor activity on the particle. In all the experiments conducted for this research, we have never seen a moving particle on a stopped microtubule. Sometimes, the particles transferred from one microtubule to another moving orthogonally. Although motion of the particles was uniformly rearward, movement was toward the (-) or (+) end of the microtubule, depending on whether dynein or kinesin was used in the assay. We have never observed that the drifting particles exhibit back-and-forth movement, described previously by Vale et al. (1989) or Okada and Hirokawa (1999). These results open possibilities for macromolecular transport/anchoring mechanisms that, although dependent on motor-driven microtubule transport, do not require direct motor attachment between the object and the microtubule. Utilized in membrane/ organelle transport *in vivo*, this mechanism could modulate motor properties such as duty cycle and processivity.

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41 Direct Observation of Protein Aggregation Induced by Specific Binding

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Conformation of protein association is of great importance to the molecular basis for biological functions. In this work, we have used cholera toxin B-oligomers (CTB) to study protein association upon specific binding to ganglioside GM1 that is incorporated in biomimetic membranes. The biomimetic membranes were prepared by vesicle fusion of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) to form supported bilayers on mica and hybrid bilayers on octadecyltrichlorosilane (OTS)-modified silicon wafer. The characteristic features of CTB-GM1 specific binding has been investigated by atomic force microscopy (AFM) under physiological conditions at molecular and submolecular resolution. This research found that, regardless of CTB and GM1 concentrations, CTB aggregates with a size of 15-20 nm formed on the supported bilayers with local crystalline structure in each aggregate, distinct from the random distribution of individual CTBs on bare mica upon nonspecific binding. On the hybrid bilayers, similar CTB aggregates were only observed on the POPC layer with ordered OTS monolayers as substrates; disordering of the OTS monolayers resulted in CTB monomers with random distribution. The results reveal the aggregation is characteristic for

CTB-GM1 specific binding, and governed by the high mobility of GM1 on the membrane surface that allowed the lateral diffusion of the CTB-GM1 complexes to form aggregates. We therefore predict that cholera aggregation may play a key role for its toxin function and immunomodulation.

Grant: LDRD Los Alamos Program

42 Molecular Nanotweezers Made of and Powered by DNA

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We have constructed a tweezer-like structure out of three strands of DNA, which consists of two double-stranded arms held together by a single-stranded region that serves as a flexible hinge. The tweezers can be successively opened and closed. The tweezers are closed via a strand of DNA called the fuel strand, which hybridizes with overhangs on the arms of the tweezers. The closed tweezers may be opened again via a strand of DNA called the removal strand. This strand is complementary to the fuel strand. The removal strand clears the tweezers of the fuel strand via branch migration. This process is initiated through the binding of the removal strand to a region of the fuel strand that remains single-stranded in the complex that consists of the tweezers and the fuel strand. Waste products consisting of the fuel strand hybridized with the removal strand are formed each time the tweezers are cycled through the open and closed states. The tweezers could serve as nanoactuators, allowing one to change the distance between two objects in a controlled manner. More generally, they illustrate a means by which nanostructures can be animated.

Electronic/Biology Interfaces

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43 Photoisomerization and Aggregation Behavior of DNA/Poly-Lysine/ Azobenzene (Dr80) Dye

Yingfan Wang, Ph.D., Eric Wallace, Mi-Kyoung Park, and Rigoberto Advincula, Ph.D., Department of Chemistry, University of Alabama at Birmingham

In this study, multilayer thin films of alternating polylysine (PDADMAC), DR80, and DNA were assembled using the alternate polyelectrolyte deposition (APD) approach based on electrostatic interaction. Organization of DNA, polypeptides, and enzymes on electrode surfaces has been previously demonstrated. Monolayers and multilayers were assembled on surfaces using polyelectrolytes as linking units. A number of potential applications have been reported, including biosensors. We have focused on the supramolecular assembly of these films as investigated by a number of surface-sensitive techniques such as ellipsometry, quartz-crystal microbalance (QCM), and atomic force microscopy (AFM). The photo-isomerization and dye-aggregation behavior under unpolarized and polarized irradiation conditions was characterized by UV-vis spectroscopy. The behavior of the dye is directly correlated to the multilayer film architecture and local dye environment, e.g., the presence of strong electrostatic interaction. Such azobenzene-containing polyelectrolyte films will be used as substrates for biosensor development in our future studies.

Grant: The Bioimplant Center, UAB

44 Electrokinetic Patterning of Cells and Beads

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We have developed a novel electrochemical system for nonlithographic, field-assisted, fluidic pick-and-place assembly of objects on a silicon substrate by means of electrical and optical addressing. The principle of our pick-and-place assembly 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. Patterning of the electrodes allows localization of charged species. This technique has previously been utilized extensively for localizing DNA. Here we first demonstrate the localization of negatively charged polystyrene beads of various sizes (0.8-20 microns in diameter) by pick-and-place assembly on patterned substrates. In addition, light-emitting diodes (50 microns in diameter) and SiO_2 pucks (100 microns in diameter) were utilized in this scheme. Finally, DNA and live mammalian cells were patterned on regular arrays by means of electrical addressing. Preliminary results indicate that cell survival was sufficient for subsequent mitosis. These results are currently being quantified further with the use of vital dyes. Furthermore, we present strategies to enhance the efficiency of cell patterning by secondary patterning of the bottom electrode with nonadhesive hydrogels. This technique has applications in creation of “active” cellular arrays for cell biology research, drug discovery, and tissue engineering.

EP5 Towards a Retinal Prosthesis: Integration of Microchannel Glass and Photosynthetic Structures into Photoelectrode Array Implants

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This presentation will describe recent progress on the integration of bioactive nanostructures into microchannel glass for the construction of a retinal prosthesis. The bioactive nanostructure is the isolated and purified Photosystem I (PS I) reaction center derived from green plant tissue. We have performed the first measurements of photovoltages (1 V) from single PS I reaction centers (Lee, et al., *J of Phys. Chem B.* 2000 104: 2439). The goal of this project is to use the PS I reaction center to build on the successful research on visual perception elicited by electrical stimulation of the retina in blind humans (Johns Hopkins University Medical School) and the development of nano- and microchannel glass, a novel material with many potentially important applications (Naval Research Laboratory). We propose to use the PS I-reaction centers, integrated onto 2-dimensional microelectrode arrays, to provide photoelectrical stimulation of the retina in blind humans. A retinal implant based on current medical implant technology would be limited to 16 stimulation channels or pixels. A visual scene based on such little information would provide a blind person with a marginal benefit. The combination of the PS I reaction center and channel-glass electrodes has the potential to instrument the retina with thousands of electrodes, each driven by a highly efficient photodetector. The metal-wire arrays are fabricated by depositing platinum throughout the hollow channels of thin wafers of nanochannel glass. The nanochannel glass provides hexagonal close-packed arrays of highly uniform channels in a glass host. The glass can be fabricated with any desired channel diameter varying from ~0.2 m to ~10 m. The platinum metal is deposited within the channels, using standard electroplating solutions and methods.

45 Integrated Microelectrode Arrays for *In Vitro* Neuronal Recording

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The objective of this research is to develop a microchip to record simultaneously from multiple neurons on a long-term basis. The microchip is fabricated by the MOSIS service using a standard 1.2 mm minimum geometry complementary metal-oxide semiconductor process. The integrated microelectrode array is comprised of an 8 x 8 matrix of 10-micron electrodes addressable via metal-oxide-semiconductor field-effect transistors. The electrodes are spaced 13 microns apart, center-to-center, with cuts in the over glass layer forming the exposed areas that are optimized for impedance in a post-processing step by sequential metallization steps that deposit Ti/Pt/Au at their tips. Signals from the electrodes pass to embedded operational amplifiers and then off-chip for subsequent data acquisition. The chip is packaged such that the active electrodes lie at the bottom of a standard cell-culture petri dish. Neurons grow readily on the array. The application of micromachining technologies should offer improved flexibility and precision in fabricating electrode arrays with superior signal-to-noise and spatial resolution. This easy-to-use and cost-effective microchip will be useful in addressing a variety of neurobiological questions.

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46 Protein-Electrode Interfaces: Understanding How Protein Structures Mediate Electron Transfer

Scott M. Reed and James E. Hutchison, Ph.D., Department of Chemistry and Materials Science Institute, University of Oregon, Eugene, Oregon

Understanding how protein structure mediates electron transfer will facilitate the design of sensors, the understanding of biological systems, and the rational construction of biomimetic nanoelectronic devices. Accurate measurement of electron transfer (ET) rates, however, requires precise control of the distance between electron donor and acceptor. Measurement of ET through peptides is therefore complicated by the inherent conformational flexibility of proteins. We have developed a peptide-containing alkanethiol thin-film model system that allows for the measurement of ET through different peptide fragments (Clegg, R.S.; Reed, S.M.; Hutchison, J.E. *J of Am. Chem. Soc.* 1998 120: 2486) whose confirmations are constrained within a molecular assembly that is covalently attached to a gold electrode. We will present evidence from x-ray photoelectron spectroscopy, infrared spectroscopy, contact angle measurements, and electrochemical experiments that these peptide-containing films possess the requisite order for accurate measurement of ET. In addition, the stabilizing influence of extensive interchain hydrogen bonding within the monolayer assemblies will be discussed. Using this system, we have demonstrated that the ET rate constant for amide material is higher than for alkanes. These results point to the possibility that protein nanostructures could be used in nanoelectronics as a mediator of electrical communication between electrodes and biological probes.

47 Dynamic Substrates for Cell Culture

Joe Tien, Ph.D., John Tan, Celeste Nelson, and Christopher S. Chen, M.D., Ph.D., Johns Hopkins University, Baltimore, Maryland

Traditional means of microfabrication are not suited to the manufacture of substrates that directly interact with living systems. Our laboratory is developing tools to improve the interface between biological and electrical elements. Because the behavior of living cells is largely determined by their local chemical and structural environments, spatial and temporal patterning of those environments may provide means to control cells at a dynamic, interactive interface. We will present advances on the spatial control of cell-chip interactions. In addition, we have recently developed approaches to fabricate structures whose properties and cellular interactions can be varied with time. We will present preliminary data on substrates that stretch, rearrange, or alter adhesivity of attached cells with time. Eventual applications include the engineered aggregation of cells into functional tissues, and the creation of adaptive biological microchips.

48 Biocompatibility of Microfabricated Neuroprosthetic Devices

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The application of prosthetic devices for treatment of nervous system disease and trauma is a practical reality. Present devices, especially those used in the central nervous system (CNS), are too large and are not completely integrated into CNS tissue. The application of microfabricated devices would result in less tissue damage, better spatial resolution, and improved electrical interaction between the device and surrounding tissue. Such devices have been fabricated with features on the micrometer scale, but their application is severely limited by the tissue's continued reactive response beginning at implantation and continuing for as long as the devices are present. A sheath of reactive cells, astrocytes and microglia, and matrix proteins forms around implantation sites. This response may have two phases: The early phase is characterized by broken blood vessels, loosely associated cells, and the beginning of sheath formation. The later phase is characterized by a dense compact sheath of cells and proteins. The development of the dense sheath generally corresponds to loss of function. The later reactive response is independent of probe size. (Probes used were 130x200, 100x100, 160x15.) Three-D microscopy of immunocytochemically labeled thick-tissue sections has permitted us to develop fabrication and pharmaceutical strategies for controlling reactive responses.

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49 Miniaturized Biosensors by *In Situ* Assembly of Colloidal Particles onto Micropatterned Electrodes

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A novel method is described for the formation of microscopic electronically readable biosensors by *in situ* assembly of functionalized latex microspheres. The active sensor patches are generated by dielectrophoretic collection of suspended particles between micropatterned electrodes. The assembly is carried out by modifying the repulsive interactions to the degree that the attractive colloidal forces coagulate the particles. Detection is carried out by secondary agglutination of colloidal gold and electroless silver deposition. This allows direct electronic readout by measuring the resistance between the electrodes. The feasibility of our approach was demonstrated by creating functional "on-chip" immunoglobulin sensors 10x30 micrometers in size. The experimental LOD of the sensors was comparable to better commercial assays and can potentially be increased much further. The method allows formation of arrays of different sensors on the same "chip." It is universally applicable to most of the latex agglutination tests available and can be extended to genetic markers (Langmuir 1999 15:3693).

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Nanotechnology in Tissue Repair

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50 Nanoscale Topography of the Corneal-Basement Membrane and Fabrication of Well-Defined Artificial Substrates with Similar Dimensions

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Basement membranes serve as substrata for overlying cellular structures and are known to influence cell behavior. The surface topographies of the corneal-basement membranes of humans, of Rhesus Macaque, of canine, and of Matrigel® (a commercially available basement- membrane-like complex) were described by TEM, SEM, and AFM. They were found to be composed of a rich, felt-like texture consisting of fibers, pores, and bumps in the nanometer range. For example, the human corneal-basement membrane has fiber diameters of 46±16 nm, pore diameters of 92±34 nm, and feature heights of 182±49 nm. Similar nanometer features also were identified in the other basement membranes studied.

Using the feature sizes described as design criteria, we fabricated and evaluated synthetic nanostructured surfaces. Our strategy is to identify salient features found on the basement membrane and evaluate each type of feature individually, using synthetic nanostructured surfaces with features of defined dimensions and periodicity. The most advanced parallel and serial nanofabrication techniques (x-ray and e-beam lithography) that are currently being developed for the microelectronics industry were used to model the basement membrane features. Fibers present on the basement membrane were modeled by ridges, holes by wells, and bumps by pillars. Biocompatibility of the surfaces is ensured by coating with a variety of materials including silicon dioxide, and self assembled monolayers (SAMs).

We have found that primary human corneal epithelial cells are sensitive to synthetic surfaces with well-defined features as small as 200 nm.

51 Application of Biocatalytic Plastics to Biomedical Implants to Reduce Biofilm Formation

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The combination of enzyme technology and polymer chemistry has led to the development of stable and active biocatalytic plastics. We describe the incorporation of the enzymes a-chymotrypsin, pronase, and trypsin into the polymeric materials Polymethyl Methacrylate, (PMMA), Polyvinyl Chloride (PVC), and Polyvinyl Acetate (PVAc).

These three enzymes were each co-dissolved in toluene/tetrahydrofuran with each of these polymers. The enzyme-polymer complexes were applied as thin coatings on surfaces such as aluminum, as well as on biomedical implant materials. Cross-linking of the enzymes with glutaraldehyde resulted in stable, entrapped enzymes within the polymeric matrices. The resultant layer was washed with bis-tris propane buffer solution, and the activity of the enzymes within the polymeric coating was measured photometrically using a chromogenic tetra peptide substrate (Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide). The enzyme quantity also was measured indirectly by using the BCA protein assay reagent at a wavelength of 562nm. Initial enzyme loading of 5 percent (w/w) resulted in 70-80 percent yield for a-chymotrypsin and 20-30 percent yield for pronase and trypsin as determined by UV spectrophotometer (280nm). Each of these enzyme-polymer complexes was then applied to materials common to biomedical implants: ultra-high molecular weight polyethylene, siloxane compounds, and polytetrafluoroethylene. These materials with a biocatalytic coating were incubated separately in culture media containing *E. coli, S. aureus*, and *C. albicans*. Biofilm development on these surfaces was analyzed at 24 hours, 48 hours, and 7 days after inoculation by scanning-electron microscopy and confocal microscopy. We conclude that these enzyme-containing polymers can be successfully applied to common biomedical implants in order to reduce biofilm formation.

52 Nanofiber Spinning and Applications of Nanofibers in Healthcare

Jayesh Doshi, Ph.D., eSpin Technologies, Inc., Chattanooga, Tennessee

Nanofibers are fibers with diameters one or two orders of magnitude smaller than conventional fibers. These fibers have a uniquely large surface area-to-mass ratio, in the range of 10 m^2/g (when the nanofiber diameter is around 500 nm) to 1000 m^2/g (when the diameter is around 50 nm). This makes them suitable for a broad range of applications. We at eSpin Technologies, a high-tech start-up company, are targeting the development of polymeric nanofiber products that are likely to revolutionize healthcare in the 21st century.

Nanofibers of biocompatible and biodegradable materials are candidates for biomedical applications such as replacement of structurally or physiologically deficient tissues and organs in humans. Use of nanofibers in tissue restoration is expected to result in an efficient, compact organ, and a rapid recovery process due to the large surface area offered by nanofibers over conventional fibers. Nanofibers made from protein may be used for the enhancement of wound healing, the epithelialization of implants, and the construction of biocompatible prostheses. Nanofibers also are candidates for solid-supported cell attachment matrices for enhancing growth. Nanofibers made from silk-like proteins could improve the blood compatibility of implanted prosthetic devices by promoting *in vitro* and/or *in vivo* epithelialization of the device, thus diminishing its thrombogenic and immunogenic properties. Scaffolding materials produced from nanofibers offer large surface areas that can support cell growth. Nanofibers also offer unique advantages in drug delivery.

53 Gene Engineered Recombinant Collagen II for Cartilage Repair

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Cartilage is an important target for tissue engineering. Attempts have been made to prepare scaffolds of natural and synthetic materials that bind and retain recombinant growth factors and scaffolds that provide for chondrocyte attachment and migration. Advances in synthesis of recombinant collagens allow for the design of materials with the most favorable properties.
This research is aimed at (1) mapping the amino-acid sequences in the collagen-II monomer that contain sites for the attachment and spreading of chondrocytes; (2) designing and testing specifically modified collagen II that can be used to prepare synthetic matrices that promote chondrocyte attachment and improve retention of growth factors; and (3) developing methods for a covalent immobilization of recombinant collagens on the surface of foam and nanofibrous scaffolds.

Using genetically engineered human collagen II variants that lack particular D-periods, we have demonstrated that the collagen-II chains of more than1000 amino acids each have a limited number of sites that promote both the binding and spreading of chondrocytes. Based on data obtained by a biosensor, we have determined that some of the analyzed growth factors attach primarily to the C-terminus of collagen-II monomers. This information will be used to design and synthesize a series of "super-collagens" that contain multiple-binding sites for chondrocytes and growth factors and therefore greatly improve their potential ability to promote cartilage synthesis.

Our preliminary results indicate that the nanofiber scaffold with covalently attached recombinant collagen-**II** monomers is an excellent support for chondrocyte attachment and migration.

54 The Role of Nano-Apatite in Mineralized Tissue Damage and Regeneration

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Our research program studies mechanisms of biomineralization via two pathways: the analysis of naturally forming mineralized structures to establish structure-function relations and the utilization of this information to develop biomimetic strategies to engineer mineralized tissue. As an example of the first pathway, we have developed insight into the coupling between mechanical and chemical changes in bone at the nanostructural level by combined nanomechanical testing with submicron chemical information attained via Raman spectroscopy. When subjected to a mechanical load, bone undergoes local pressure-induced phase transformations, exemplified by shifts of phosphate u1 peaks and peak broadening. This latter result supports the hypothesis that amorphization of bone occurs in a similar manner to that observed in synthetic apatites. As an example of the second research pathway, we have orchestrated nucleation and growth of a continuous bone-like carbonated nano-apatite on the interior pore surfaces of functionalized 3D porous polymers via a one-step biomineralization process. Following deposition of the nanostructured biomineral, moduli increased threefold. Furthermore, the benign processing conditions (i.e., room temperature, atmospheric pressure) enable incorporation of recombinant factors into both the mineral and polymer, facilitating a number of permutations of controlled release. A secondary effect of this self-mineralization strategy is that the nano-apatite also can serve to buffer scaffold degradation products and enhance osteoblast function and bone tissue engineering. The biological significance of this approach is demonstrated by significant changes in alkaline phosphatase, collagen, and collagen and osteocalcin mRNA levels with small, transient shifts (e.g., 0.2) in pH.

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55 Nanostructured, Surface-Phase Separated Structures Created in Blends of Linear and Hyperbranched Polymers

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Blends of a hyperbranched polyester (HBP) having alkane end groups tend to phase separate to a fiber surface when melt blended with a linear polymer such as polyethylene. The resultant fiber has a surface containing ca. 50nm diameter droplets that contain pure hyperbranched polymer. The objective of our study is to determine why this happens and how it can be manipulated for improved implant materials and tissue scaffolds through manipulation of their surfaces.

The blends were prepared by dry mixing the two solid polymer powders together. Then they were extruded through a heated single-screw extruder (like an Archimedean screw) to produce the unique nanophase-separated structure within a 1mm diameter fiber. The extrusion is critical in producing the resultant morphology. The fiber's water-contact angle is significantly increased to 90 degrees, making the surface extremely hydrophobic. The pure polyethylene fiber has a water-contact angle of ca. 84 degrees, while the pure HBP has a water contact angle of 108 degrees. We will show how these HBPs affect the surface of poly(lactic acid) and other polymeric fibers through measuring the contact angle and nanoscale surface roughness.

56 Phase Separation in Poly-Pseudo Amino Acid-PEG Blends and Copolymers at Nano- and Meso-Length Scales

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Such properties as hydrophilicity, hydrolytic susceptibility, and protein recognition are critical to the success of polymer-based tissue-engineering scaffolds. Poly(ethylene glycol) [PEG] is often introduced into scaffold polymers to tune hydrolytically controlled properties. 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 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. Experiments and simulations of fibronectin adsorption onto different homopolymer thin-film surfaces suggest that nano- and mesoscale phase separation may influence the adsorption of such adhesive proteins and, in turn, have an impact on cell adhesion and proliferation.

57 Micro-Engineering Cartilage Tissue Scaffolds

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Aim of Study: We are developing tissue scaffolds of precise biomimetic architecture to serve as support and confinement structures for chondrocytes and for fixation of cartilage matrix molecules.

Methods: Auto Cad software was used to design templates for square arrays of pseudolacunae. The size of the arrays ranged between 15 x 15 and 100 x 100 units. These templates were used as specifications in the fabrication of a lithographic mask. Mask fabrication was followed by plasma etching of a 3” silicon wafer. Aqueous agarose was then applied to the silicon template and allowed to gel. Finally, the agarose cast was peeled from the template, inverted, and placed in a 100 mm petri dish. The depth of the pseudolacunae was 50 microns. Suspensions of chondrocytes were then applied to these scaffolds with and without fibronectin coating. Nonpatterned substrates were used as controls.

Results: The combination of fibronectin coating with micropatterning greatly increased cell adhesion and, ultimately, matrix production from the constructs. Over several days of growth, progressive accumulation of matrix was readily seen and significantly exceeded matrix production on nonpatterned control substrates. By varying the dimensions of the microstructures, we were able to control the number of cells in each cluster, permitting us to create an array of isolated growth units that contain single cells or small groups of cells.

Conclusions: Micropatterned scaffolds hold significant promise for cartilage tissue engineering. Advances in this technique will include nanoscale channels between lacunae in order to facilitate cell signaling and matrix attachment.

58 Nanoparticle Resin Composites with Low Shrinkage, Radiopacity and Translucency

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Most dental restorative resins are replaced due to secondary caries, primarily the result of shrinkage during curing, and esthetic composites are not radiopaque. To overcome these problems, resin composites with low cure-shrinkage and monodispersible, radiopaque, surface-grafted, metal-oxide nanoparticle fillers were investigated. The low-shrinkage monomers are liquid-crystal (LC) dimethacrylate monomers. The nanoparticle fillers are 10-200nm metal oxides.

1,4(4-(6-methacryloyloxy-hexane-1-oxy)benzoyloxy)2-(t-butyl)benzene [C6(H,tBu,H) was synthesized and formulated to form visible light-curable resins. Cure shrinkage was determined, without reinforcing filler, with an automated mercury dilatometer and compared to commercial restoratives (e.g., Z-100, 3M Dental Products).

Mono-dispersed, zirconia (ZrO_2) nanoparticles (avg. = 11 nm) were prepared by a non-aqueous sol/gel process, and surface-grafted with silanes and methacrylate zirconium coupling agents. These were co-dissolved with a blend of bis-GMA, TEGDMA, and bis-EMA (GTE) and photo-polymerized to maximum degree of double-bond conversion (DC). Controls were Z-100 and the unfilled GTE. DC, flexural strength, modulus, fracture toughness, and wear resistance were determined.

The unfilled liquid crystal resins shrink 2.3 vol. percent ± 0.3, not different ($p<0.05$) from highly filled resins (1.8 percent ± 0.2). The zirconia-filled specimens have excellent handling properties and high translucency. Radiopacity of 30 wt. percent nanocomposites is 1.9 times aluminum ~enamel). Strength and modulus are ≥ unfilled GTE.

Thus LC monomers reduce cure shrinkage by ~75 percent, and ZrO_2 nanoparticles can be homogeneously dispersed and improve properties of bis-GMA based resins. However, their maximum loading is insufficient to affect wear resistance. Therefore, continued effort is needed to realize further improvements.

59 Polyurethane Nanocomposites: New Materials for Biomedical Applications

James Runt, Ph.D.[1], Richard Xu, Ph.D.[1], Christopher Siedlecki, Ph.D.[2], and Evangelos Manias, Ph.D.[1]

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[2]Department of Surgery, Pennsylvania State University, State College, Pennsylvania

Poly(urethaneurea)-segmented block copolymers [PUU] are used in variety of biomedical applications, most prominently as blood sacs in ventricular-assist devices and total artificial hearts. However, one of the principle drawbacks of these biocompatible materials is their relatively high permeability to air and water vapor. In this presentation, we will describe a nanocomposite approach that results in a significant reduction in gas permeability through PUUs, without sacrificing mechanical properties. In initial experiments, PUU (22 wt percent hard segment) / alkyl-ammonium modified montmorillonite nanocomposites were prepared containing low-volume fractions of the layered silicate (<6 vol percent). X-ray diffraction experiments show that the silicate gallery spacing increases by about 1 nm for most of the composites, indicating that PUU chains are intercalated to some degree between silicate layers. At very low silicate concentrations, the layers may in fact be exfoliated. The measured modulus and strength increase with increasing silicate content in the nanocomposites, but without loss of ductility. Water-vapor permeability is reduced by 5x at only 6 vol percent silicate, as a result of the more tortuous path required for gas molecules to penetrate the membrane. We also plan to discuss issues regarding these materials in blood-contacting applications, particularly with regard to protein adsorption to the separated microphases.

60 Nanostructured Diamond Coatings for Dental TMJ Implants

Yogesh K. Vohra, Ph.D.[1], Marc D. Fries[2], William R. Lacefield, Ph.D.[3], Jack E. Lemons[3], and Krishna Venugopalan, Ph.D.[4]

[1]Department of Physics
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[4]Depatment of Biomedical Engineering, University of Alabama at Birmingham

The Departments of Physics, Dental Biomaterials, and Biomedical Engineering at the University of Alabama at Birmingham (UAB) will present their interdisciplinary study to evaluate nanostructured diamond coatings for dental implants. We test the hypothesis that the wear characteristics of TMJ (temporomandibular joint) implant devices can be improved by manipulation of the nanostructure of diamond coatings (adhesion and wear). Nanocrystalline hard and soft diamond coatings are produced by a microwave plasma chemical vapor deposition process with a new chemistry based on methane/hydrogen/nitrogen species. Varying the nitrogen concentration in the plasma during growth can control the hardness of the coatings from 10 GPa (soft) to 100 GPa (hard) while retaining the ultra-smooth surface (roughness of 14 nanometers). Nanoindentation studies have been carried out on hard and soft surfaces to measure the modulus and hardness of coatings as well as wear tolerance under simulated dental conditions. Bulk indentation up to 150 kg load with a 1/8 inch tungsten carbide ball shows no film delamination. *In vivo* biocompatibility testing of nanostructured diamond and calcium phosphate ceramics are planned to be carried out, using standardized model systems , including injection of particulate debris, soft and hard tissue evaluations, and functional joint replacement to simulate anticipated human TMJ conditions for 6 and 12 month comparisons of articulation and fixation.

61 Nanoceramics: Novel Formulations of Biomaterials with Unique Properties for Orthopaedic/Dental Applications

Thomas J. Webster, Ph.D., Richard W. Siegel, Ph.D., and Rena Bizios, Ph.D., Rensselaer Polytechnic Institute, Troy, New York

Often, clinical complications with conventional orthopaedic/dental implant devices composed of metal and metal alloys are due to insufficient bonding to juxtaposed bone. Ceramics have long been appreciated for their biocompatibility with bone cells and tissue; poor mechanical properties (such as ductility) have, however, limited their wide use as orthopaedic/dental implants.

This study demonstrated that, compared to conventional (grain sizes greater than 100 nm) formulations, nanophase ceramics (specifically, alumina, titania, and hydroxyapatite) exhibited bending properties on the same order of magnitude as physiological bone. Such properties are highly desirable for materials used as bone implants. Most important, the functions (such as adhesion, proliferation, and deposition of calcium-containing mineral) of osteoblasts (the bone-forming cells) were significantly enhanced, while adhesion of fibroblasts (cells that contribute to fibrous encapsulation and callus formation events that lead to implant loosening and failure) were minimized on all nanophase ceramics tested.

Investigation of the mechanism(s) of the observed, select, enhanced osteoblast adhesion (a crucial prerequisite for subsequent, anchorage-dependent-cell function) on all ceramic formulations tested in the present study revealed that the concentration, conformation, and bioactivity of vitronectin were important parameters mediating osteoblast adhesion exclusively on nanoceramics.

By demonstrating that bioceramics can be designed and fabricated (through control of grain size) to possess improved mechanical properties that promote select osteoblast function, the results of the present study demonstrated for the first time that nanophase ceramics have the potential to become the next-generation preferred orthopaedic/dental biomaterial to enhance bonding to juxtaposed bone and, thus, to increase implant efficacy.

62 Nanofiber Mat with Capability to Deliver Bioactive Compounds

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We are developing ultra-fine fiber polymeric mats (permanent or biodegradable) with a capability of releasing bioactive compounds. These mats can be stand-alone or can serve as film coatings on implants, tissue engineering scaffolds, or nanocomposites. To construct the drug-loaded mats, various concentrations of finely ground fluorescently labeled Bovine Serum Album (FITC-BSA) are suspended in 25 wt percent Polylactic Glycolic Acid biodegradable polymer in 50:50 dimethyl formamide:tetrahydrofuran. Suspensions contained in a glass syringe with a capillary tip were spun into ~500nm diameter fibers by an electrostatic-based self assembly process (electrospinning), in which a high voltage electric field was generated between the oppositely charged polymer and a metallic collection screen. At a critical voltage, the charge overcame the surface tension of the deformed polymer drop at the needle tip, producing an ultrafine jet. The similarly charged fibers were splayed, and during their passage to the screen, the solvent quickly evaporated and dry fibers accumulated randomly on the screen. Material properties of the nonwoven mesh mats are investigated by SEM and tensile testing. *In vitro* release of the model protein (FITC-BSA) into an infinite sink of 37ºC phosphate-buffered saline (mimic *in vivo* conditions) is measured. Preliminary results indicate that tensile strength and the release profiles are a function of protein loading. Although release in the first 24 hours after initiation is dominant, release to over 120 hours is observed. The preliminary data suggest that this nanofiber delivery system can open up many new applications in both drug delivery and tissue engineering.

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Synthesis of Biomimetically Derived and Bioactive Nanostructures

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63 Antimicrobial Dendrimer-Silver Complexes and Nanocomposites

Lajos Balogh, Ph.D.[1], D.A. Tomalia, Ph.D.[1], Gary L. Hagnauer, Ph.D.[2], and Albert McManus, M.D.[3]

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The use of silver as an antimicrobial agent has been recognized for some time. It is an effective agent with low toxicity, which is especially important in the topical antibacterial treatment of burn wounds, in which transient bacteremia is commonly cited.

Surface-modified poly-amidoamine (PAMAM) dendrimers were utilized as templates/nanoreactors/ containers to pre-organize silver ions and subsequently form nanosized, silver containing hybrid particles which are solubilized, stabile, and have a high surface area yet are nearly uniform. These silver dendrimer nanocomposites (DNC) have formed external type nanosized composite materials as witnessed by their NMR, UV-*vis* spectroscopy, and high resolution TEM images (*J of Nanoparticle Res,* 1999 1(3): 353-368).

Different {Ag(0)-PAMAM} dendrimer complexes and silver nanocomposite solutions were tested *in vitro* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using the standard agar overlay method. Final concentrations of the samples were confirmed by atomic absorption spectroscopy. The protected silver salts and dendrimer silver nanocomposites displayed considerable antimicrobial activity without the loss of solubility/activity even in the presence of sulfate or chloride ions. Both the silver-dendrimer complexes and the nanocomposites displayed antimicrobial activity comparable to, or better than, those of silver nitrate solutions. Several DNC agents dramatically outperformed the 10 percent silver nitrate solution used as a control. However, if common cellulose membranes are used, diffusion of dendrimer nanocomposites can be totally stopped.

In this presentation, further medical application potentials of silver nanoscopic devices will also be discussed.

Grant: DAAL 01-96-2-0044

64 Dendrimer Nanocomposites

Lajos Balogh, Ph.D., Center for Biologic Nanotechnology, University of Michigan, Ann Arbor, Michigan

Dendrimer nanocomposites (DNC) are recently discovered nanosized hybrid materials (*Chemical Innovation* March 2000: 19-26) that display unique physical and chemical properties. The goal of this presentation is to demonstrate the principles and application potentials for this novel group of nanomaterials.

In their synthesis, dendrimers are used as templates to pre-organize small molecules or metal ions followed by an *in situ* reaction immobilizing atomic or molecular domains of various guests. The atomic/molecular level dispersion of inorganic guest(s) within a dendrimer host is achieved by reactive encapsulation. Size, shape, size distribution, and surface functionality of these stable nanocomposites are determined and controlled by the dendritic host. The solubility and compatibility of these materials are also determined by the host polymer molecule; however, these nanocomposites possess many of the desirable chemical and physical properties of the guest molecules or atoms. For example, precipitation of metallic gold into the interior of a poly(amidoamine) dendrimer results in the formation of a gold dendrimer nanocomposite. This material has the solubility of the host dendrimer but possesses the optical and physical properties of the guest nanoscopic gold domains.

These novel materials have many potential applications for bioengineering and medicine because the properties of both the guest(s) and the host can be optimized to form uniform basic structures with required characteristics. These building blocks can be organized further into higher order structures, such as 1D (quantum dots), 2D (ultrathin multilayers), and 3D nanostructures to achieve a specific goal by combining the tools of polymer, organic, inorganic, and bioorganic chemistry.

65 Porosified Silicon Wafer Structures as Drug Delivery Systems for Chemotherapy

Jeffery L. Coffer, Ph.D. and Xin Li, Department of Chemistry, Texas Christian University, Fort Worth, Texas

We have successfully developed a process that permits the encapsulation of the established class of platinum anticancer drugs such as cis-platin (cis-diammine-dichloroplatinum [II]) and carbo-platin (cis-diammine [cyclobutane-1,1-dicarboxylato]) within synthetic biocompatible calcium phosphate films that are electrochemically grown on nanoporous silicon/silicon (Si/Si) substrates. The underlying silicon substrates are compliant with existing microelectronics processing methods. Upon immersion of these structures into aqueous media, the desired platinum species can be released into the surrounding environment. These platinum complex-doped hydroxyapatite/ porous Si/Si materials have been characterized by scanning electron microscopy, energy dispersive x-ray spectroscopy, and secondary ion mass spectrometry.

In this presentation, we focus on the influence of initial platinum concentration for a given complex in the deposition process on the rate and resultant amount of platinum complex that can be delivered to the surroundings *in vitro*. Both inductively coupled plasma spectroscopy as well as UV-visible absorption spectrometry have been employed to monitor the release of the encapsulated drug from the calcium phosphate layers. The impact of subsequent thermal annealing of the calcium phosphate/ porous Si/Si structure on the rate of complex delivery to the surroundings will also be discussed.

66 Toward Biomimetic Methods of Crystal Synthesis

James J. De Yoreo, Ph.D.[1], Patricia M. Dove, Ph.D.[2], Christine A. Orme, Ph.D.[1], Hui H. Teng, Ph.D.[3], and Aleksandr Noy, Ph.D.[1]

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By deterministically modifying the nucleation, kinetics, morphologies, and facet stabilities of crystals, many organisms produce nanophase materials as well as complex single-crystals and multilayer composites. A physical understanding of these biomineralization processes can lead to development of biomimetic methods for synthesis of nanostructured materials and strategies for addressing disease in mineralized tissue. Because $CaCO_3$, in association with acidic amino acids, is ubiquitous amongst biomineralized structures, we are examining the nucleation and growth of $CaCO_3$ single crystals in the presence of acidic amino acids. Here we report results of *in situ* atomic force microscopy investigations of growth, as well as nucleation on carboxyl-, methyl-, and hydroxyl-terminated self-assembled monolayers (SAMs). Using measurements of the dynamics of atomic steps on the crystal surface, we determined the changes in thermodynamic and kinetic factors induced by the organic fraction. Introduction of the amino acids led to little or no change in growth kinetics but dramatically altered both crystal morphology and the magnitude and orientational dependence of the surface energy. The new crystal symmetry depended upon the chirality of the amino acid, showing that the binding of the amino acid to the crystal involved both the chiral center and multiple sites on the calcite surface. Finally, nucleation of $CaCO_3$ on patterned SAMs occurred exclusively on the carboxyl-terminated regions, showing that the surface functional groups altered the interfacial energy. These results lead naturally to a hypothesis for biomineral growth that invokes modification of surface energies through adsorption of proteins and peptides.

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DOE, Divison of Geosciences and Engineering Contract No. DE-FG0298ER14843

67 Virus Protein Cages: Encapsulation of Materials through Design

Trevor Douglas, Ph.D.[1], Mark Young, Ph.D.[2], Erica Strable[1], Sue Brumfield[2], Debbie Willets[2], Aleksey Nedolushko, Ph.D.[1], Therese Sterner[1], and Tom Arnold[2]

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The native protein shell of the plant virus Cowpea Chlorotic Mottle Virus has previously been shown to act as a constrained reaction environment for the encapsulation of inorganic and organic species. The virus capsid is comprised of 180 protein subunits that self-assemble to form particles. The icosahedral symmetry has an interior cavity of about 20 nm in diameter and can be assembled into an empty protein cage devoid of nucleic acid.

Recent work using site-directed mutagenesis of the protein has allowed us to alter, by design, the physical characteristics of the protein cage to accommodate a range of functions aimed at materials synthesis as well as at therapeutic and diagnostic applications. These alterations to the protein cage include: modification of 180 intrinsic Ca-binding sites to accommodate Gd binding for MRI applications; incorporation of fusion proteins for cellular targeting; alteration of protein electrostatics to accommodate encapsulation; and synthesis of size-constrained materials from fluorescent nanoparticles of CdS to magnetic Fe-oxides. We have taken a biomimetic approach, making minimal changes to the existing system with no apparent disruption of the viral capsid.

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68 Biomolecular Recognition of Semiconductor, Optical, and Magnetic Materials

Christine E. Flynn, Erin E. Gooch, Jamie K. Schaeffer, Sandra R. Whaley, and Angela M. Belcher, Ph.D., University of Texas at Austin

We are developing biologically inspired techniques for the patterning and interconnecting of electronic and magnetic materials on nanolength scales in order to design and investigate next-generation materials and devices for electronic and medical applications. A peptide combinatorial approach has been employed to identify proteins that select for, and specifically bind to, inorganic materials such as semiconductor quantum dots. The approach utilizes the inherent self-organizing, highly selective properties of biologically derived molecules. Because nature has not had the opportunity to produce biomolecular interactions with some of the desired materials, phage display libraries have been used to evolutionarily select peptides that will bind to these materials. We have sequenced and cloned peptides en route to developing genetically engineered proteins with higher affinity binding. A range of inorganic substrates has been investigated, including GaAs, GaN, Fe_3O_4, ZnS, Si, $CaCO_3$, and $LiNbO_3$. Some peptides have demonstrated face specificity, size control, and crystal phase discrimination. Putative consensus sequences and binding epitopes have been mapped, allowing for analysis of the chemical driving forces of these interactions. Positive binding peptides were characterized for substrate recognition, discrimination, and binding, using fluorescence, spectroscopy, and microscopy. These peptides will be used to nucleate and pattern useful electronic, optical, and magnetic materials.

Grant: ARO # DAAD 199910155

69 Fabrication of Nano-Dimensional Honeycombed Ceramic Thin Films of Controlled Pore Size

Craig A. Grimes, Ph.D., University of Kentucky, Lexington, Kentucky

The objective of our work is to fabricate thin-film honeycombed ceramic layers of controlled nano-dimensional pore size on ferromagnetic magnetoelastic films. The nano-dimensional honeycomb structure offers a tremendous surface area for sensing, for storage of liquids such as insulin, and can serve as a template for further material growth. The ferromagnetic magnetoelastic film can be made to vibrate in response to an AC magnetic field, thereby controlling the flow of liquids or gases into or out of the honeycombed ceramic structure.

Using sol-gel techniques, nano-dimensional honeycomb-structured Al_2O_3 and TiO_2 ceramic layers have been deposited on amorphous, ferromagnetic thick films. We believe that the honeycomb structure is achieved due to the highly charged sol particle, as it comes out of solution, following the stray magnetic field associated with a domain wall of the ferromagnetic film. An analogy would be the ionized particles following earth's magnetic field, resulting in the Aurora Borealis about the North Pole. The size of the honeycomb pore is determined by the size of the magnetic domain upon which the sol solution is coated, which can be controlled by application of a DC magnetic field.

70 Structure and Mechanical Properties Studies on the DEJ for Biomimicry of an Interface

Stefan Habelitz, Ph.D.[1], Sally J. Marshall, Ph.D.[1], Grayson W. Marshall Jr., Ph.D., D.D.S., M.P.H.[1], and Mehdi Balooch, Ph.D.[2]

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The dentin-enamel junction (DEJ) unites two dissimilar calcified tissues, hard and brittle enamel, and softer and tougher dentin. It is remarkably successful in protecting the biomechanical integrity of the tooth, because cracks initiated in enamel often are deflected or limited by the DEJ and do not continue to propagate into dentin and through the entire tooth.

Human third molars were studied in the area of the enamel and the DEJ using atomic force microscopy combined with nanoindentation in order to characterize structure and site-specific properties. Elasticity and hardness of enamel were found to be a function of the microstructural texture. Mean Young`s moduli of 93.6 (± 2.3) and 77.8 (± 4.7) GPa and mean hardness values of 4.4 (± 0.4) and 3.8 (± 0.4) GPa were determined in directions parallel and perpendicular to the enamel rods respectively. Intertubular dentin showed isotropic elastic modulus and hardness of 21 (± 1.3) GPa and 0.5 (± 0.02) GPa, respectively. Nanoindentations were performed along lines of 50 mm length encompassing the DEJ, enamel, and dentin at intervals of 1 or 2 mm. Within the DEJ region, both elastic modulus and hardness showed a monotonic gradation from bulk values of enamel to dentin. Estimates of the functional width of the DEJ based on its nanoproperties were 11-12 mm, which was much broader than its microscopic appearance. At loads of 6000 mN, indentation cracks were initiated in the enamel close to the DEJ but did not penetrate the interface.

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71 Electron Transport Properties of Metal Nanoparticles Chemically Assembled on Biomolecular Scaffolds

James E. Hutchison, Ph.D., Department of Chemistry, University of Oregon, Eugene, Oregon, and Martin N. Wybourne, Ph.D., Department of Physics and Astronomy, Dartmouth College, Hanover, New Hampshire

The novel electronic properties (e.g., Coulomb blockade) of nanometer-scale assemblies of metal nanoparticles make them potentially useful as electrically addressable, extremely sensitive chemosensors. To date, no straightforward and reproducible methods are available for the fabrication and interconnection of low-dimensional nanoparticle assemblies. Our methods for nanofabrication of linear nanoparticle arrays is a biomimetic approach involving self-assembly of functionalized metal nanoparticles onto rigid biomolecular scaffolds bridged between narrowly spaced electrodes. Our previous investigations of unpatterned nanoparticle thin films provided clear evidence of Coulomb blockade at room temperature, but the response was unstable over time. In this paper, we present synthetic methods, scanning probe microscopic and surface analytical investigations, and electron transport studies of arrays of gold nanoparticles assembled onto a polypeptide (poly-L-lysine) scaffold layer. The assembly process provides a simple, chemical method to immobilize the particles and is found to stabilize the electrical response (Coulomb blockade) of the array compared with unpatterned samples. Results from electrical measurements suggest that the electronic properties of these arrays are remarkably tolerant of defects and disorder, an important prerequisite for applications in electronic or sensing applications. A possible application of these self-assembled devices in diagnostics is the detection of biologically relevant molecules at or near single molecule detection limits.

Grant: NSF-DMR-9705343

72 Generation of Molecular-Gradients of Self-Assembled Monolayers Using Microfluidics

Noo Li Jeon, Ph.D., Stephan K.W. Dertinger, Ph.D., Insung S. Choi, Ph.D., Daniel T. Chiu, Ph.D., Abraham D. Stroock, Ph.D., and George M. Whitesides, Ph.D., *Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts*

This poster describes a simple, versatile method of generating molecular-gradients of mixed self-assembled monolayers (SAMs) on surfaces using a microfluidic device. This method is based on controlled diffusive mixing of self-assembling molecules in solutions flowing laminarly, at low Reynolds number, inside a network of microchannels. We demonstrate the use of this procedure to generate gradients in surface ydrophobicity-hydrophilicity and gradients in surface resistance to protein adsorption. The gradients examined here went from maximum to minimum values over lateral dimensions of ~150 um; they were formed from steps in concentrations with lateral dimensions of < 10 um. Gradients of different size, resolution, and shape have been generated using this method. The method is experimentally simple, highly adaptable, and requires no special equipment except for an elastomeric relief structure that can be prepared readily by rapid prototyping. The microfluidic gradient generator provides a new platform with which to study phenomena that depend on gradients in surface properties, especially dynamic phenomena in cell biology (haptotaxis) and surface chemistry (nucleation and growth, etching, and Marangoni effects), using well-controlled microenvironments.

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73 Metal Nanoparticles Modified with Molecular Receptors on their Surfaces

Jian Liu, Julio Alvarez, and Angel E. Kaifer, Center for Supramolecular Science and Department of Chemistry, University of Miami, Coral Gables, Florida

The surfaces of metal cluster and colloidal particles can be derivatized with molecular receptors. The properties of these nanoscopic systems can thus be controlled using the molecular recognition properties of the surface-attached receptors or hosts. For instance, we have modified gold colloidal particles (diameter: 13 nm) with cyclodextrins and taken advantage of their host properties to control colloidal flocculation rates through host-guest interactions with solution guests. We have also prepared smaller gold, platinum, and palladium nanoparticles (diameters in the range 2.5-15 nm) capped with monolayers of thiolated cyclodextrins. We have used electrochemical and nuclear magnetic resonance techniques to show that the particle-attached cyclodextrin hosts actively engage in molecular recognition interactions with appropriate guests in the solution. The palladium and platinum nanoparticles are water-soluble and exhibit catalytic activity for hydrogenation reactions. These systems currently are under investigation as novel catalysts for "green chemistry" applications. Some preliminary results obtained with other classes of hosts and their potential applications as sensors and catalysts will also be discussed.

74 Synthetic Macromolecular Systems with Secondary and Tertiary Organizations: Designing Nanostructured Biofunctional Macromolecular Systems

Ishrat M. Khan, Ph.D., Lisandra J. Ortiz, Ph.D., Sandra Thorpe, Keith Gordon, and Kariyawasam Pemawansa, Ph.D., Department of Chemistry, Clark Atlanta University, Atlanta, Georgia

The overall goal of our studies is to develop methods by which nanoscale macromolecular assemblies with biofunctional properties may be readily prepared. Our approach involves both synthesis of helical macromolecules and integration of helical structures into tertiary structures. We expect our studies to result in the development of facile methods for preparing nanoscale macromolecular assemblies or complexes with tertiary structures and enzyme-like biospecific properties.

A facile method for preparing helical poly(3-methyl-4-vinylpyridine) will be reported. Because of the conformational organization of this functional macromolecule, poly(3-methyl-4-vinylpyridine) may be utilized as building blocks for nanostructured functional materials with application in synthetic biomembranes, synthetic enzymes, drug delivery systems, and tissue engineering. The preparation and characterization of these helical macromolecules and some of their macromolecular assemblies will be reported.

The preparation of synthetic biofunctional material requires the assembly of biospecific peptides, e.g., with specific amino acid sequence for binding to cell-surface proteins, into a tertiary structure that would permit the biospecific properties and functions to be maintained. Here we will report on two methods that permit the formation of such organized tertiary structure containing secondary helical polypeptide chains. Using the dehydration of reverse micelles method, macromolecular complexes of sodium poly (a,L-glutamate) (PGNA) [MW 1K, 49K and 71K] and ethylene oxide-propylene oxide block [PEO-PPO-PEO] triblock copolymers with specific tertiary organization may be prepared. The tertiary organization is composed of a helical PGNA core with spiral wound PEO blocks connected via a PPO segment. The secondary and tertiary structures of the macromolecular complexes were determined by 2-D NOESY, differential scanning calorimetry (DSC) and CD spectroscopy. The methods of preparation and characterization details will be discussed.

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75 Development of "Active" Nanostructures with an Icosahedral Virus

Tianwei Lin, Anne Struble, Qian Wang, M.G. Finn, and John E. Johnson, Department of Molecular Biology and Chemistry, Scripps Research Institute, San Diego, California

Cowpea mosaic virus (CPMV) is an icosahedral plant virus with a size of 30nm in diameter and can be produced in gram quantities. Our previous studies of this virus by X-ray crystallography and molecular virology led to the development of a chimeric virus technology with which foreign peptides were incorporated into the virus capsid. These peptides adopted a variety of conformations and bestowed the virus particles with exogenous properties. We are extending this technology to develop CPMV as active components of biomaterials. Exogenous properties of green fluorescence and metal ion binding are being introduced into the capsid by presenting anchoring peptides on the CPMV surface so that full-length green fluorescence protein can be attached and introduce His residues based on the geometry of residues found in metal ion binding proteins. Optimal constructs are to be made by application of evolutionary pressure in combinatorial approaches. In a separate study, we demonstrated that CPMV could be prepared as self-assembled nanostructures of 3D arrays. Of particular interest is a 3D array in a hexagonal space group, which could bind exogenous proteins and form virus/protein complexes *in situ* within the array. In the near future, we will combine the chimeric virus technology with the production of virus arrays, in hopes of producing new sensing biomaterials displaying optical or enzyme amplified readouts, among many possible nanostructures.

76 Nanofabrication of Protein Multilayers by Alternate Adsorption with Polyions: From Planar Films to Assembly on Microtemplates

Yuri Lvov, Ph.D., Institute for Micromanufacturing, Louisiana Tech University, Ruston, Louisiana

Layer-by-layer assembly of molecularly organized films was developed for polyions and proteins. Neutron and X-ray reflectivity, scanning electron microscopy, QCM, AFM, and UV-absorbance were used for studying the film structure.

Protein architecture: Multilayer film growth by alternation of linear polyions and 20 different proteins was elaborated. Protein/polyion multilayer assembly provides new possibilities in the line of efforts to create a method of organizing proteins in layers and to build up such layers following "molecular architecture" plans. The method was extended to build up multiprotein films (i.e., alternate arrays of different protein monolayers) in predetermined orders.

Neutron and x-ray reflectivity curves were measured for layered films of proteins and deuterated polyions. Multilayers of myoglobin (Mb) or glucose oxidase were prepared in alternation with deuterated or hydrogenated polystyrenesulfonate (PSS-H and PSS-D) and poly(ethyleneimine). Analysis of these curves gives a thickness of Mb/PSS bilayer of 5.6 nm. A contrast introduced for neutron scattering by deuterated PSS resulted in formation of Bragg peak at Q = 0.06 A-1, which indicates spatial separation of PSS-D layers in myoglobin film in the case of quaternary unit cell.

Enzymatic activity in multilayers: Enzymatic activity of glucose oxidase, glucoamylase, peroxidase, alkaline phosphatase and carbonic anhydrase was preserved in multilayers with linear polyions and increased with the number of layers at least up to 10 protein monolayers.

Sequential enzymatic reaction: Multienzymatic sequential reactions with directed transfer of products were realized for glucoamylase/ glucose oxidase/ peroxidase catalyses.

Bio/nanoreactor: The enzyme multilayer assembly on 300-nm diameter latex provides tiny catalytic units stable in aqueous solutions. The enzymes in such units are active and are protected by polyion immobilization.

EP6 Biomimetic Materials for Molecular Analysis

Terry A. Michalske, Sandia National Laboratories, Albuquerque, New Mexico

Miniaturized chemical analysis systems are expected to play an important role in health care by providing rapid, low-cost analysis for complex biomolecular mixtures. Although the overall architectures for these devices will involve micron-scale flow systems, the functions of molecular sorting, recognition, and detection will be revolutionized by designing nanostructured materials that mimic biological detection and molecular recognition processes. Unfortunately, most biological or biomimetic structures are simply too fragile to be integrated within microfabricated, chip-based devices. We are developing a new class of materials by co-assembling organic membranes within inorganic scaffold architectures. The membranes are designed to carry out molecular recognition and detection while the inorganic scaffold provides a robust material that can be directly integrated with chip-based microfluidic systems. We have demonstrated this new approach to biomimetic materials with membranes including lipid bilayers and polydiacetylene vesicles that are self-assembled within meso-scale silicate hosts. Signal transduction for molecular recognition is tranduced either through optical or electrochemical signatures.

77 Membrane-Mimetic Architectures at Mesoporous Thin Films

Atul N. Parikh, Bob Provencal, Theotis Clark, Andy Shreve, Jeff Brinker, and Basil Swanson, Los Alamos National Laboratory, Los Alamos, New Mexico

We show that mesoporous thin films provide a useful support for phospholipid bilayers for the study of cell membrane processes and to interface membrane mimetic architectures to transducer surfaces. Mesoporous thin films of controlled thicknesses in the 100-1000 nm range, displaying a cubic array of 6-10 nm-size water-filled pore channels, were formed on silicon, glass, and gold substrates by directing the surfactant-templated self-assembly of sol-gel precursors at the substrate surfaces. Photochemical removal of the surfactant templates rendered the substrate surfaces hydrophilic and the pore channels water-filled under aqueous media facilitating facile fusion of phospholipid vesicles to form continuous supported bilayers. The water-filled pore channels of the mesoporous support further provide a tenacious aqueous cushion that serves to decouple local substrate-chemistries from the lipid bilayers. Characterization of the bilayer structure, fluidity, and homogeneity using fluorescence recovery after photobleaching, atomic force microscopy, and Fourier-transform infrared vibrational spectroscopy revealed the stuctural integrity of the bilayer films. Further exploitation of the surface-pore channels to localize well-hydrated transmembrane proteins within the supported bilayer matrix currently is being explored in our laboratory, along with the application of these membrane architectures to sensing protein toxins.

78 Growth of Mammalian Cells on Micropatterned, Hyperbranched Polymer Thin Films

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Here we describe a four-step soft-lithographic process based on microcontact printing (μCP) of organic monolayers, hyperbranched polymer grafting, and subsequent polymer functionalization, which result in polymer/n-alkanethiol patterns that direct the growth and migration of mammalian cells. The functional units on these surfaces are 3Dimensional cell "corrals" that have walls 52±2 nm in height and lateral dimensions on the order of 60 μm. The corrals have hydrophobic, n-alkanethiol bottoms that promote cell adhesion, and walls consisting of hydrophilic poly (acrylic acid)/poly (ethylene glycol) layered nanocomposites that inhibit cell growth. Fourier transform infrared-external reflection spectroscopy, ellipsometry, contact-angle measurements, and X-ray photoelectron spectroscopy (XPS) reported previously confirm PAA grafting and PEG functionalization. Tapping-mode atomic-force microscopy and optical microscopy provide proof of polymer patterning. These films were robust and could be steam- or ultraviolet light-sterilized without delaminating from the substrate. Cells seeded on patterned surfaces adhere and grow within the corrals, but they do not span the PAA/PEG corral walls. Cell viability studies indicate that cells remain viable on the patterned surfaces for as long as 21 days, and fluorescence microscopy studies of stained cells demonstrate that cell growth and spreading does not occur outside of the corral boundaries. This simple, chemically flexible micropatterning method provides spatial control over growth of peritoneal macrophages, endothelial cells, and hepatocytes. We are presently using mammalian cells confined in high-density arrays such as these as sensors for high-through-put screening. They may also find applications for tissue engineering, particularly if these methods can be translated to biodegradable substrates.

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79 Synthesis and Interactions of Monodispersed Drugs and Semiconductors in Medical Applications

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Monodispersed particles, of sizes as small as several nanometers, have found many uses in modern biotechnology and medicine. We present our research program on preparation and characterization of uniform suspensions of organic and inorganic particles, with applications in synthesis of drugs, interactions of biological materials with uniform inorganic particles, and adhesion of particles on model biological surfaces.

With the exception of polymer latex suspensions, little is known about formation of uniform organic dispersions. Our research program has focused on procedures for obtaining monodispersed particles of pure organic compounds. By manipulating solubility properties, and by changing solvents and pH, we control particle size and porosity of finely dispersed drugs.

For diagnostic purposes, we have developed techniques for depositing biomaterials onto inorganic particles, such as proteins on metal oxides. The resulting coated particles react chemically as proteins but exhibit new magnetic conductance and other physical properties. In contrast, nanosize particles can adsorb on much larger biological species, e.g., cells. For example, semiconductor CdS quantum dots of a few nanometers exhibit useful fluorescence properties for cell labeling.

We demonstrate that packed column techniques allow quantitative evaluation of deposition and removal of inorganic particles at organic surfaces. Glass spheres coated by proteins have been used as substrates. For small depositing particles, the adhesion is due to direct interactions with the surface. It is possible to ascertain conditions for adhesion by varying pH, ionic strength, and other properties.

EP7 Biomimetics: From Biology to Technology

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Structural control of inorganic materials at the nanometer scale is a key to synthesis of materials with new and improved physical properties. Biological hard tissues may serve as models for novel engineered materials, as biocomposites have excellent combinations of physical properties that are related to their highly ordered hierarchical structures. The intricate nano- and micro-architecture of biocomposites are controlled at the molecular level by macromolecules through interactions with mineral phases. In this presentation, human and mouse enamel, mollusc shells, and sponge spicules will be discussed as biocomposite examples that offer materials science and engineering lessons for functional materials assemblies (piezoelectric, mechanical, and optical). The central issue, mimicking of biological structures, requires the use of macromolecules, in particular proteins that have affinity to inorganic surfaces. Although proteins can be isolated from biological tissues, a more practical strategy is to use genetic engineering techniques to develop novel non-natural proteins with high affinity to inorganic surfaces. The second part of the presentation will cover combinatorial genetic techniques that permit isolation of specific recognition elements for inorganic surfaces, including those not realized by natural proteins, in the absence of a priori prediction of necessary structures. The results could have significant implications in tailoring surfaces and in the formation and assembly of ordered structures of metals, functional ceramics, semiconductors, and ferroelectrics in applications of nanotechnology, smart materials, bioimplants, and biomimetics.

80 Directed Neuron Attachment and Outgrowth on Patterned Topographic and Chemical Clues

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Neurons possess a highly complicated geometry that is the result of numerous developmental and function-dependent signals. We are using microscale patterns of chemical signals and topographic features to study processes controlling axon and dendrite growth and the development of synaptic function. Simple patterns are used to permit analysis of individual hippocampal neurons. Topographic signals used are pillar arrays in which both pillar diameters and interpillar distances are controlled. Axon growth is stimulated by growth on these surfaces. Axon-growth characteristics are controlled by the geometry of the patterns. Patterns of biologically active proteins are being made using photolithographic procedures and microcontact printing. Patterns of poly-lysine and signaling molecules, such as laminin, are being used to direct neuron attachment and axon growth. Overlays of chemical signals on topographically modified surfaces are also under investigation. Neuron attachment and axon and dendrite growth are monitored in live cells and assessed using immunocytochemistry, fluorescence and confocal microscopy, and SEM. Features of patterns are aligned to extracellular electrodes fabricated in the single-crystal silicon or glass base of culture/recording chambers. Quantification of axon and dendrite growth and electrophysiology is being used to assess control of neuron growth and development.

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81 Fabrication of Nanostructured Interfaces Between Bioactive Glass and Ti Alloys

E. Saiz, J. M.Gomez-Vega, and A.P. Tomsia, Materials Science Division, Lawrence Berkeley National Laboratory, Berkeley, California

The objective of this work is to prepare nanostructured interfaces between silicate glasses and Ti alloys that will allow the fabrication of bioactive coatings with optimum adhesion to metallic implants. Using a conventional enameling technique, titanium alloys were coated with a new family of bioactive silicate glasses. The softening points and thermal expansions of the glasses permitted the fabrication of coatings without generating large thermal stresses. The glass/metal interface was characterized using x-ray diffraction (XRD), scanning electron microscopy (SEM), and high-resolution transmission electron microscopy (HRTEM), combined with energy dispersive spectroscopy (EDS). The adhesion was evaluated using Vickers indentation. Finally, the *in vitro* behavior of the coatings in simulated body fluid (SBF) was also analyzed. Coatings with optimum adhesion were prepared by the controlled formation of an interfacial nanostructured Ti_5Si_3 layer, ~150 nm thick. The layer is divided into two regions: a continuous nanocrystalline layer in contact with the alloy and, on top of it, a zone with isolated Ti_5Si_3 nanoparticles dispersed in the glass. During *in vitro* tests in SBF, apatite precipitated on those coatings with surface silica contents lower than 60 wt percent. The apatite grows in the form of oriented nanocrystals with the c-axis perpendicular to the substrate. The crystals incorporate 1 to 5 wt percent MgO in their structure, substituting for CaO. In conclusion, the controlled formation of a nanostructured interface permits the fabrication of glass coatings on Ti alloys able to precipitate apatite during *in vitro* tests and with excellent adhesion to the metal.

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82 Role of Mechanical Forces in Fibronectin Self-Assembly and Biorecognition

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Although major progress has been made in the past to reveal how chemical factors regulate biorecognition, insight into pathways by which nature utilizes external forces to regulate biorecognition and signaling holds the potential for major new discoveries in biomedicine. Knowledge in this field is rudimentary since high-resolution crystallographic structures of biomolecules have been obtained mainly from equilibrated states. The role played by mechanical forces applied to the terminal ends of a protein in regulating self-assembly and biorecognition events will be discussed here for the multidomain protein fibronectin. One of the many functions of fibronectin is to promote cell adhesion to surfaces. External forces are crucial to initiate its assembly into fibrillar matrices on the surface of cells, and a model system was established to identify critical intermediates in the fibril assembly pathway. Starting from the equilibrium structure of fibronectin domains, steered molecular dynamics simulations were applied to study the pathway by which their tertiary structure unravels under external forces, and major differences have been found in their mechanical stability. Finally, external force can also have a major impact on regulating biorecognition events as illustrated here for the exposure of its RGD-loop to integrins.

83 Peptoid-Based Foldamers: Application of Synthetic, Sequence-Specific Polymers for Biological Mimicry of the Helical Lung Surfactant Proteins SP-B and SP-C

Cindy W. Wu and Annelise E. Barron, Ph.D., *Department of Chemical Engineering, Northwestern University, Evanston, Illinois*

Foldamers are non-natural, sequence-specific oligomers designed to mimic the folding properties of natural polypeptides. We are designing protein mimics that are based on a class of synthetic, sequence-specific foldamers called “polypeptoids” (i.e., N-substituted glycine polymers), whose advantages for biological mimicry include their ease of synthesis, protease stability, and low immunogenicity. Using automated solid-phase synthesis, we produce polypeptoids with specific sequences of biomimetic, proteinogenic sidechains. Despite an absence of chiral centers and hydrogen-bond donors in the polymer backbone, certain polypeptoid sequences adopt stable helices in solution that exhibit intense circular dichroism (CD) spectra resembling those of peptide alpha-helices.

Along with studying the fundamental folding propensities of biomimetic polypeptoid molecules, we have focused on designing peptoid-based mimics of the helical lung-surfactant proteins SP-B and SP-C, as safe, bioavailable alternatives to animal-derived surfactant replacements that are useful for the treatment of Respiratory Distress Syndrome (RDS) in prematurely-born infants. Whereas natural SP-C peptide misfolds and aggregates in solution, peptoid-based, helical SP mimics are stable in solution and show promising biophysical properties as determined by CD, surfactometry, and surface fluorescence microscopy.

Grant: NSF BES-9870386

84 Triplet Oxygen Enhancement of ^{13}C NMR Relaxation of Surface and Bulk Spins of Calcite

James P. Yesinowski, Materials Chemistry Branch, Naval Research Laboratory, Washington, DC

Improved techniques for characterizing the structure of solid surfaces of nanoparticles at the atomic level are needed. Solid-state nuclear magnetic resonance (NMR) spectroscopy can provide detailed chemical and structural information but is not inherently a surface-selective technique. This poster presents an experimentally simple approach to improve both the *selectivity* as well as the *sensitivity* of surface NMR based upon the accelerated relaxation of surface spins by their interaction with paramagnetic triplet oxygen molecules (Surface-Enhanced NMR Achieved by Triplet Oxygen Relaxation or SENATOR). The results also demonstrate improved detection sensitivity for nuclear spins in the bulk nanocrystalline solid.

Calcite, a stable polymorph of calcium carbonate, was chosen for study because of its major role as a biomineral (e.g., in egg and sea shells). The natural-abundance static ^{13}C NMR signal at 11.7 T displays an axially symmetric chemical-shift anisotropy powder pattern of 77 ppm; all carbon atoms in the lattice are identical and have the same shielding-tensor orientation. The ^{13}C spin-lattice relaxation times T_1 of a high-specific surface area (70 m^2/g) rhombohedral calcite sample have been measured under a variety of temperature and oxygen partial pressure conditions (from 173K to 298K and from 0 to 1Mpa, respectively). One sample contained only natural abundance ^{13}C; the other was labeled with ^{13}C at the surface by gas-phase exchange with $^{13}CO_2$. In all cases, the saturation-recovery curves exhibit stretched-exponential behavior $M_z(t) = M_0(1\text{-}e(-[t/T_1]^{ß})$, with signal arising from both surface spins as well as subsurface spins in the interior of the nanocrystals. By taking advantage of the different spin-spin relaxation times T_2 of surface ^{13}C-labeled spins and interior spins in a Hahn-echo pulse sequence, it is possible to observe the spin-lattice relaxation behavior of both pools of spins separately. Enhanced spin-lattice relaxation of the ^{13}C surface spins by triplet oxygen is observed, especially at lower temperatures or higher oxygen pressures, and this relaxation enhancement is transferred to interior spins via spin diffusion.

These experiments demonstrate that triplet oxygen is an experimentally convenient, relatively nonreactive, and readily removable relaxation reagent that allows NMR spectra from surface spins to be obtained at higher repetition rates (increased sensitivity for a given time). When spin diffusion into the interior of nanoparticles is present, NMR spectra of subsurface spins can also be obtained with increased sensitivity. A reduction by an order of magnitude in the time required has been obtained in the present experiments, even under non-optimal conditions. The SENATOR technique should be useful in probing the interaction with the mineral surface of peptides that alter the crystal growth and habit of calcite; such studies are currently underway.

85 Imparting Biomimetic and Biological Recognition Properties to Interfaces with Organic Shell-Structured Nanoparticle Network Film

Chuan-Jian Zhong, Felicia X. Zhang, Mathew M. Maye, Jason G. Daras, Li Han, Yongbin Lou, Nam K. Ly, and Lisa G. Israel, Department of Chemistry, State University of New York at Binghamton

This presentation describes recent findings of biomimetic and biological recognition properties of nanostructured interfaces using core-shell nanoparticles. The recognition properties are derived from organic open frameworks in the network nanostructures as nanochannels or nanosites. Nanometer-sized gold and alloy particles of different core sizes (2 to 5 nm) and their geometric arrangements define the size and shape, whereas thiolate shell structures define the chemical and biological specificity. Thin films of such nanostructures are fabricated and they are electronically conductive, specularly reflective, and biocompatible. Biomimetic ion-channel properties are demonstrated by measuring pH- and potential-tuned responses to charged redox and ionic probes. Spectrophotometric selective determination of biologically relevant thiols is demonstrated using the nanostructured particles and assemblies. New ways of immobilizing biological molecules at the nanostructures will be explored via novel interfacial reactivities at the interparticle linkages. Infrared reflectance spectroscopy and scanning probe microscopy (scanning tunneling and atomic-force microscopies) are employed to characterize the interfacial morphologies, reactivities, and structures. The results will be discussed in terms of potential applications for developing spectroscopic immunoassay and electrochemical biosensors. These findings present intriguing new pathways to biomedical explorations via designing core-shell nanoparticle architectures at both nanocrystal and molecular levels.

Grants: ACS-PRF 35550-G5
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Exhibit Abstracts

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EX1 Digital Instruments BioScope

Jeff Duran, Ph.D., Veeco Micrology Group, Digital Instruments, Santa Barbara, California

The Digital Instruments BioScope is an atomic force microscope (AFM) designed specifically for the biosciences. It incorporates our industry-leading AFM design with a specially modified inverted optical microscope to create a familiar platform for AFM work. The BioScope stage also provides rigid mounts for standard petri dishes, microscope slides, and cover slips, simplifying sample mounting and requiring little or no sample preparation.

AFM is a breakthrough technology that allows 3-D imaging from the angstrom to micron scales, allowing researchers to visualize the atomic and molecular structure of materials. In its most basic form, AFM images topography by precisely scanning a small probe (several nanometers in end radius) across the sample to "feel" the contours of the surface. In addition, AFM can be used as an ultrasensitive force probe (sensitivities in the piconewton range) capable of measuring molecular elasticity, interatomic/molecular bonds, Van der Waals forces, adhesion, hydration forces, hydrophobic forces, electrostatics, etc.

AFM has allowed researchers in the biosciences to visualize and measure native biological structures (i.e., no staining and/or coating is required) at extremely high resolution. The ability to obtain this high resolution in either air or fluid under ambient conditions has opened a whole new window on the workings of biomolecules, membranes, etc. Time-resolved studies of the function of biological structures under physiological conditions have helped researchers in their quest to understand structure/function relationships. As previously mentioned, the AFM also can be used as an ultra-sensitive force sensor capable of detecting single ligand-receptor unbinding events, or it can be used to study a wide variety of other biological applications (e.g., force-induced conformational change of bacteriorhodopsin at 10Å lateral and 1Å vertical resolution). Published examples of AFM's utility in the biosciences include:

- DNA, protein, and chromatin structures
- Enzyme/substrate interactions
- Protein/protein, protein/DNA interactions
- Adsorption properties of cells and molecules to biological or other surfaces
- Macromolecular assemblies
- Cell surface antigens
- Cellular and molecular interactions
- Antigen/antibody complexes
- Cell morphology and motility
- Measurement of mechanically induced unfolding or single proteins
- Dynamic variation in chemical and mechanical properties of biological surfaces
- Synaptic release and signal transduction processes
- Planar membranes and liposomes
- Viruses, bacteria, and other microbes
- Biomaterials
- Real-time, *in situ* monitoring of events at biological surfaces

An extensive bibliography of recent publications regarding the use of AFM in the biological sciences can be viewed by visiting our web site at http://www.di.com. Click on *Bibliographies and Related Sites* and then *Bibliographies: Biological Sciences*. Several researchers at NIH have already realized the potential of AFM, while others are just beginning to explore its power. This is an important technology for Nanoscience and Nanotechnology that should be available to all that participate in this important symposium.

EX2 Mac Mode™ Atomic Force Microscope: Applications and Recent Developments in Biological Research

George Sibbald, Molecular Imaging, Phoenix, Arizona

Because of its high spatial resolution (sub-nanometer) and ability to image in fluid under controlled environment (e.g., 37^{o}C), the atomic force microscope (AFM) is quickly becoming a routine tool for scientists in various fields. Molecular Imaging's PicoSPM line of AFMs is uniquely designed for special imaging conditions, such as various buffers, temperatures (both heating and cooling), as well as controlled gases. Our innovative product, the magnetic AC mode (MAC Mode™) AFM, provides the highest imaging resolution (less than 1 nm) of soft biological samples in fluid.

With the recent developments in tip modification, such as antibody attachment, the application of MAC Mode™ AFM is expanded beyond imaging. When modified with an antibody, the AFM tip could locate a specific antigen on the sample surface. This technique potentially can become an important tool in identifying membrane proteins such as receptors and channels in the cell membrane.

Our new areas of interest include the study of protein folding/unfolding by pulling the protein with the AFM tip, attaching a nanotube to the end of conventional tip to generate high aspect ratio tip, and continuously improving sample preparation procedure. By applying ‘pulling’ force on a molecule such as titin, one can unfold and refold the 3D structure, and further measure the unfolding force down to piconewtons. When combined with an inverted microscope, the MAC Mode™ AFM becomes the first high-resolution AFM imaging tool with single molecule fluorescence capabilities. For more information, application notes or newsletters, see us on the web at www.molec.com.

EX3 QCM-D Measurements of Biomolecule and Surface Interactions

Patrik Dahlqvist, Q-Sense AB, Gothenburg, Sweden

Biological substances in contact with solid, non-biological materials, is a situation of broad scientific interest and technological importance, and there is a growing need for new tools to study these interactions. Among many different properties of the biological films formed at the interfaces, the visco-elastic properties are of central interest, since these properties can be used as a discriminator in bio sensing and in the study of polymer films.

We have developed a sensor system based on the traditional quartz crystal micro balance (QCM) technique, but where both the resonant frequency (*f*) and the energy dissipation ? *D*) are measured simultaneously for a non-driven (freely oscillating) sensor crystal. This provides accurate and precise measurements of *f* and *D* in the gaseous and liquid phases.

This study investigated the process of lipid membrane formation on solid supports, followed by streptavidin functionalisation and attachment of oligonucleotides for real-time investigations of hybridisation processes.

EX4 High Speed Imaging of Single Molecule Fluorescence Using Cooled CCD Camera Systems

Mark Christenson, Ph.D., Roper Scientific, Tucson, Arizona

This poster presents cooled CCD-camera systems that are capable of imaging single molecule fluorescence at rates from sub-video (15 Hz) up to ultra-high speed (15 kHz). This can be accomplished with intensified cameras or back-illuminated cameras. With the I-PentaMAX-intensified camera, the net gain boosts the signal far above the system noise, permitting single photoelectron detection capability. Using standard-mode operation, frame rates from 15 Hz to 100 Hz can be obtained. Using a new Virtual Chip mode of operation, imaging at rates from 100 Hz to 1000 Hz can be obtained. Data will be shown of single molecule imaging at kiloherz rates.

With a back-illuminated camera system, the detectability of a signal is limited by the readout noise of the camera. This noise is an exponential function of readout speed, suggesting that high-speed imaging is not feasible with a back-illuminated camera. We demonstrate two modes of operation through which this limit can be bypassed. The first method uses the Virtual Chip mode, in which data can be collected at 10 Hz up to 300 Hz. The second method uses kinetics mode, in which a burst of images is collected on the CCD and the image set is then read out slowly. In kinetics, we are able to achieve over 15,000 Hz.

These camera technologies are all currently available for use in single molecule fluorescence imaging and are supported within a variety of hardware and software environments.

EX5 Nanofabrication Users Network

Gregory T. Baxter, Ph.D., National Nanofabrication Users Network, Cornell University, Ithaca, New York

The National Nanofabrication Users Network (NNUN) is a National Science Foundation-funded consortium of five university facilities, Cornell University, Stanford University, Pennsylvania State University, Howard University, and the University of California at Santa Barbara. These facilities are complementary in equipment and technology and serve users from both industry and academia. This partnership is dedicated to providing high precision processing equipment in sophisticated facilities, supported by expert personnel and the latest in computer design aids. The combination of facilities, equipment, and expertise provided by the members of the Network is unsurpassed in any single research laboratory. The consortium supports research in the areas of biology, electronics, materials, MEMS, optoelectronics, physics, and process characterization. The NNUN was formed as a partnership of nanofabrication user facilities, providing unparalleled opportunities for creative people to turn new ideas into experimental reality. Looking ahead to the next decade, an explosion of valuable new applications for nano- and microfabrication is evident. The ability to manipulate the structure of materials, with a precision approaching atomic dimensions, will be used in new electronic devices and in structures that impact a range of research disciplines from physics to biology.

EX6 Nanostructured Chemicals: A New Era for Polymeric Nanocomposites and Biomaterials

Joseph D. Lichtenhan, Hybrid Plastics, Fountain Valley, California

This exhibit provides an overview of an emerging class of nanostructured chemical technology that offers scientists and engineers the ability to upgrade the physical properties of conventional materials in a "turn-key fashion" through the use of existing manufacturing practices.

Polyhedral Oligomeric Silsesquioxane (POSS™) chemical systems represent a unique union of hybrid (inorganic/organic) materials and nanocomposite technology. POSS™ technology is the only commercially available nanostructure for chemical incorporation into polymeric and bio-based materials. POSS™ chemical technology is unique in that its composition is a hybrid (intermediate between polymers and ceramics), and POSS™ structures are nanoscopic and nearly equivalent in size to most polymer dimensions. Hence, POSS™ chemical systems may be viewed as the smallest chemically discrete particles of silica, possible while the resins in which they are incorporated may be viewed as silica-reinforced nanocomposites.

POSS™ technology is produced commercially in bulk and is available in a highly diverse number of POSS™ reagents, fillers, monomers, polymers, and surface modification agents, thereby affording unprecedented new chemical technology for the design of materials with improved and unique physical properties.

POSS™ technology is easily incorporated into existing material formulations using conventional polymerization, grafting, or compounding protocols. Furthermore, their compatibility with existing polymers, ceramics, and biological systems can be controlled through the manipulation of the solubilizing/nonreactive organic groups located on the cages.

The recent commercialization of Nanostructured POSS™ chemical feedstocks has fueled tremendous interest in academic and industrial research and development programs geared toward the development of materials having both hybrid and nanocomposite properties. Technical advantages, limitations, economic aspects, product applications and emerging markets for Nanoreinforced Plastics based on POSS™-chemical technology will be presented. Specific biomaterials and bioresearch directions will be described.

EX7 Nanoparticle Delivery Systems (NDS)

S. Mohan, Ph.D., PolyMicrospheres, Indianapolis, Indiana

PolyMicrospheres, widely recognized for designing and developing nanosphere and microsphere based drug delivery systems, remains at the forefront of microencapsulation and other matrix technologies. We design and develop nanoparticle and microparticle delivery systems with embedded chemotherapeutic and other drugs for the controlled delivery of drugs preferentially to affected tissues over an extended period of days to weeks. We develop antibody and other moiety-bound Nanoparticle Delivery Systems (NDS) embedded with chemotherapeutic drugs to achieve targeted and extended delivery to cancer cells. We also develop surface-modified NDS for better cellular penetration and distribution of the nanospheres in the tumor cells.

Although there are several potent anticancer drugs currently on the market, their use is limited because of toxicity to normal tissues. By microencapsulating drugs in nanosphere matrices, we can control the release of drugs without reaching toxic levels. By further targeting the drugs to be concentrated in the tumor tissue, we can selectively reduce toxicity with a concurrent increase in therapeutic index.

The NDS offers the advantage of site-specific or targeted delivery of chemotherapeutic and other drugs to affected cells over an extended period, thereby increasing efficacy while reducing toxic side effects.

We use many biodegradable and/or biocompatible polymers and develop delivery systems with particle sizes ranging from 40 nm to 1000 µM. We work with water-insoluble and water-soluble drugs for various applications. We develop NDS to provide optimum nanoparticle size and surface properties to be targeted to and to accumulate into the affected tissues to achieve maximum cellular penetration, to ensure adequate drug loading for a continuous drug release from days to weeks, and to achieve maximum activity with minimum toxicity.

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