between cells. Metropolis Monte Carlo (MMC) simulations based on DAH predicted the emergence of long-lived structures (cell sheets, toroidal and tubular constructs) in accord with experiments. The MMC method, however, does not describe time evolution. We propose a kinetic Monte Carlo (KMC) approach, where transition rates are associated with possible rearrangements of cells. The system is represented on a lattice, with sites occupied either by cells or by volume elements of cell culture medium. We associate rates to swapping cells with nearest neighbors of different types (cells or medium). The new approach was tested against experiments on cell sorting within an aggregate composed of two cell types. In quantitative studies, we determined the time evolution of the interfacial area between two fusing spherical cell aggregates experimentally, analytically and by KMC simulations. In the analytic approach, we used continuum hydrodynamics to describe the coalescence of two identical, highly viscous liquid droplets, and obtained good agreement with experiments on smooth muscle cell aggregates. Apart from the early stages of fusion, the KMC method predicted a fusion pattern similar to the experimental one. Comparison with measurements allowed relating KMC transition rates to experimental time scales. Our results indicate that the KMC method can give an accurate account of the time evolution of complex cellular structures, thus it may be a useful tool for tissue engineering applications. Work supported by NSF-056854.

3269-Pos Board B316

Fully Biological Bioprinted Blood Vessel Substitutes

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Cardiovascular disease is a leading cause of death and often requires vascular reconstruction. There is considerable clinical need for alternatives to the autologous vein and artery tissues used for vascular reconstructive surgeries, lower limb bypass, arteriovenous shunts and repair of congenital defects to the pulmonary outflow tract. Engineering new tissues, ideally from the patient's own body cells to prevent rejection by the immune system, is a rapidly growing field that rests on three pillars: cells, supporting structures (or scaffold) and stimulating biological environment. However the use of scaffolds has often been associated with chronic inflammation and impaired tissue-remodeling and maturation. In this respect understanding the physical principles of biological self-assembly is essential for developing efficient strategies to build living tissues and organs. Here we exploit well-established developmental processes (such as tissue fusion, spreading or sorting phenomena) to engineer small-diameter blood vessels. We introduce a novel automated rapid prototyping method (bioprinting) that allows the building of three-dimensional custom-shaped tissue and organ modules without the use of any scaffold, thus making the final construct fully biological, as well as structurally and functionally closer to native tissues. Conveniently prepared bio-ink units (multicellular spheroids or cylinders composed of single or several cell types) are delivered into the bio-paper (a hydrogel support material) to build linear and branching tubular structures of small diameter (down to 0.9 mm OD). Structure formation takes place by the post-printing fusion of the discrete units. Upon removal of the support material, the fused construct is matured in perfusion bioreactor under pulsatile flow until desirable biomechanical (burst pressure, compliance) and biochemical (e.g. ECM) properties develop. Such constructs could fulfill the crucial need for small diameter vascular grafts and provide new strategies for vascularization of tissues for transplantation.

3270-Pos Board B317

Amphiphilic Peptides That Self Assemble into Nanomicelles and Vesicles

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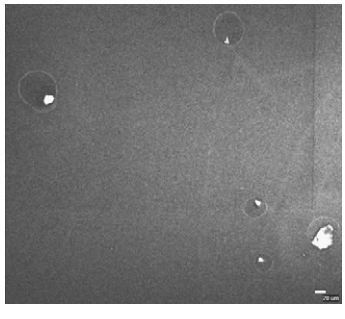
Self-Assembling peptide nanovesicles are attractive candidates for drug delivery. Here we report the characterization of specific amphiphilic peptides (up to 23-amino acids in length) that undergo supramolecular assembly to form both mono- and hetero-assemblies that form nanomicelles and larger vesicles (150nm) in aqueous solutions. Depending on the peptide's composition and the pH of the aqueous medium during assembly, the peptides adopt either a micellar or vesicular structure. Simple amphipathic sequences adopt a micellar structure (<50nm) at low to neutral pH. When pairs of lyophilized peptides with different lengths are co-dissolved in an unbuffered Carboxyfluorescein solution at a 1:1 molar ratio, they self-assemble into small vesicles that are visualized using a confocal microscope. These intact vesicles average about 150nm in size (as determined by confocal images) and are capable of entrapping solutes. CD and FTIR analyses of such mixtures indicated a tendency of the peptide to adopt a beta sheet secondary structure. Isothermal Titration Calorimetry (ITC) revealed the Critical Aggregation Concentration (CAC) for the individual peptides to be less than 1mM, which is a useful property during drug delivery. These nanostructures can be used as models for further developments.



3271-Pos Board B318

High Throughput Lipid Bilayer Technologies

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Measurements of ion channels are important for scientific, sensing and pharmaceutical applications. Reconstitution of ion channels into lipid vesicles and planar lipid bilayers for measurement at the single molecule level is a laborious and slow process incompatible with the high throughput methods and equipment used for sensing and drug discovery. A recently published method of lipid bilayer formation mechanically combines lipid monolayers self-assembled at the interfaces of aqueous and apolar phases. We have expanded on this method by vertically orienting these phases and using gravity as the driving force to combine the monolayers. As this method only requires fluid dispensation, it is trivially integrated with high throughput automated liquid-handling robotics. In a proof-of-concept demonstration, we created over 2200 lipid bilayers in three hours. We show single molecule measurements of technologically and physiologically relevant ion channels incorporated into lipid bilayers formed with this method.

3272-Pos Board B319

Construction of a Bioprinted Fully Biological Nerve Graft

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Annually over 200,000 peripheral nerve surgeries are performed in the U.S. alone. Many of these procedures require grafts to bridge the severed nerve. Autologous nerve is the gold standard for providing a temporary support across which axons regenerate. Although successful at 80%, the autograft procedure has several drawbacks including the limited number of available nerves, the loss of function and /or sensation at the donor site and the need of multiple surgeries. Synthetic or natural nerve guidance hollow tubes have been used successfully as scaffolds for small gaps (<3cm) but regeneration fails over longer distances. As a result, tissue engineering has emerged as a promising alternative. Based on recent studies, synthetic and autologous tubes failure has been linked to low density of supporting cells such as Schwann cells and the lack of longitudinally-oriented structural features, which favor Bunger's bands-like formation and axonal growth by mimicking endoneural architecture. In addition, axonal growth can be impaired by inflammatory and immunological responses triggered by the implanted scaffold.

We present here a novel tissue engineering technology that is based on principles of developmental biology and employs bioprinting, This automated rapid prototyping method allows for creating well-defined architectural features, without any scaffold, thus making the final construct completely biological as well as structurally and functionally closer to native tissues. Spherical or cylindrical bio-ink units (composed of Schwann and bone marrow stem cells) are delivered according to a computer scrypt together with agarose rods, as support material units. Structure formation takes place by the post-printing fusion of the discrete units. The geometrical parameters of those tubes, such as wall thickness, diameter, and number of lumens can easily be controlled. Such constructs could fulfill the crucial need for larger nerve grafts.

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3273-Pos Board B320

Influenza A Nucleoprotein Detection by a Novel Immuno-Interferometric Sensor

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Rapid detection and identification is imperative to combat known or emerging infectious agents. This novel immuno-interferometric sensor utilizes the specificity and selectivity of antibody-antigen interactions to detect and identify a specific influenza viral component in a label-free manner. Primary antibodies to the nucleoprotein of Influenza A (IFA) were oriented utilizing a previously reported polymer- protein interaction system of poly(methyl methacrylate) (PMMA, CAS# 9011-14-7) and biolinker protein G′. This unique noncovalent adsorption method resulted in increased primary antibody orientation, and

enhanced assay sensitivity. Indeed, the immuno fluorescence assay was found to have a detection limit of 1.25 μg/ml of IFA nucleoprotein. Combining this novel antibody orientation method and Fabry Perot (FP) Interferometry, a PMMA-on-silica label-free biosensor was designed and fabricated. A 100nm PMMA layer was spin coated onto a 1.5 μm silicon dioxide wafer. Illuminating and receptor fibers were set at 45° and 135° angles to measure the reflected spectra. Protein G', Anti-Influenza Primary Antibody, and Influenza Antigen were added to the sensor surface incubated, washed and air-blown dry prior to measurements. Sequential addition of protein G′, primary antibody, and viral antigen to the sensor surface were concurrently verified by non-contact AFM imaging analysis. Spectrum demodulation, known for high resolution and accuracy, was employed to process total thickness changes of the PMMA-silicon chip. The determined subsequent spectral shifts correlated with binding of detector proteins and ultimately influenza to sensor surface. This novel sensor, as an analytical tool, has the potential to quickly and easily detect influenza and other biohazards. *Supported by NSF Grant EEC-0425826 and Army Research Office Grant W911NF-07-02-0081*

3274-Pos Board B321

A Novel Self-Assembled, Self-Healing, Ordered Biomaterial

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Geometrically-ordered biomaterials are beneficial to tissue engineering and to studies of cell mechanics. In addition, techniques to produce microscale and nanoscale ordered objects can be applied to solve engineering problems in industrial settings. We present a magnetically self-assembled two-dimensional crystal of thrombin-coated superparamagnetic microbeads formed on a liquid-air interface. The thrombin-coated beads catalyzed the cleavage of fibrinogen in solution to form fibrin fibers that self-assembled into a nanometer-scale fibrin network whose fibers have been shown to follow the ordering of the scaffolding bead crystal (Alsberg, et al. (2006) Tissue Engineering. 12, 3247.). Computer simulations and analysis of confocal fluorescence microscopic images reveal the mechanism by which the system self-assembles to its geometrically-ordered form. We demonstrate that the process of self-assembly is dependent on the lattice geometry, but not on the details of fibrin biology. We formulate a set of rules that are required for ordering: (i) The monomers must be adherent to the scaffold beads, (ii) The monomers must be able to polymerize into a linear polymer in solution, (iii) Growing polymer fibers must be able to diffuse about a pivot at their point of attachment to the beads and (iv) Interactions between the monomers, polymers, and fibers, other than polymerization must be minimal. We demonstrate a microrheological system for measuring the time-dependent dynamics of formation of the fibrin network. The crystalline order of the magnetic microbeads allows us to sample the forming network's viscoelastic properties at regularly spaced intervals. Using the magnetic interactions between the beads as a force transducer, we demonstrate the effects of mechanically perturbing the forming gel, showing that broken connections in the network are repaired. Taken together, the results suggest that the process is generalizable, and that the resulting system is self-healing during the formation of the lattice.

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Pisep, A Powerful New Ion Exchange Chromatography Using Controlled Ph Gradients For Separating Proteins On Anionic And Cationic Stationary Phases

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pISep is a new low ionic strength IEX. It consists of externally controlled pH gradients over the pH range from 2 to 12 created by the mixing of two specific cocktails of small organic buffers, one at an acid pH, the other at a basic pH. Gradients can be generated on strong or weak cationic or anionic exchangers over arbitrary pH ranges wherein the stationary phases remain totally charged. Software makes possible the calculation of accurate linear, nonlinear or combined, multi-step, multi-slope pH gradients. An extension of the pISep technology enables the formation of fully controlled, externally generated pH gradients in the presence of additives such as NaCl (up to 1.0 M). The ability to add salt while retaining control over the formation of pH gradients provides much improved flexibility for the separation of proteins sensitive to extremes of pH. Further extensions of the method include the formation of fully controllable pH gradients in the presence of up to 8M urea or 80% acetonitrile. Subsequent creation of mathematical manifolds that define the mixing proportions of the acidic and basic buffers to attain a specific pH as a function of both pH and additive concentration has allowed the creation of software to create simultaneous independent gradients of pH and either salt, urea, or acetonitrile. This latter technology amounts to two dimensional chromatography on a single column. We present examples here of very high resolution separations of model proteins, hemoglobins, MAbs, whole cell extracts, and trypsin digests of BSA , demonstrating the wide versatility of the methodology. Extensions of the electrostatic theory of protein binding to charged stationary phases will also be discussed in light of the experimental results reported here.

3276-Pos Board B323

Development Of Novel Biomimetic Membrane Designs For Separation And Biosensor Applications

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Integral membrane proteins have a variety of functions e.g. as channels, transporters or receptors. In order to study transmembrane proteins under controlled circumstances they must be embedded into a matrix mimicking their *in vivo* environment. There has been a growing interest in developing a biomimetic platform technology for biosensor and separation applications.

Current design criteria for free-spanning artificial membrane platform technologies are low leak membrane sealing, membrane stabilities above 1 day, absolute reproducibility, a scaffold consisting of multiple functional units, enablement of reconstitution of membrane spanning molecules, be robust for transportation and cost effective. For mass transfer flow and high throughput screening applications additional design criteria are required. These include a high perforation level of the membrane scaffold material, the functional membrane units are arranged in arrays to facilitate a screening platform (e.g. for microplate readers) and the artificial membrane platform is scalable to met various requirements for the individual technical applications.

Recently, we have developed a model biomimetic membrane design and an automation technique for establishing multiple black lipid membranes (BLMs) in arrays of micro structured ethylene tetrafluoroethylene films, and supported by a micro porous material. Success rates for establishment of supported BLMs across multiple aperture arrays were above 95%.

Currently, work is focused on characterization of nanoporous materials and surface modifications together with different lipid compositions. Furthermore, to develop methods for the encapsulation of established membranes and for the controlled incorporation and distribution of transmembrane proteins into such encapsulated biomimetic membranes. Combined this work aims to meet all of the current design criteria for free-spanning artificial membrane platforms.

3277-Pos Board B324

Silk/silica Nanocomposites As Novel Biomaterials For Tissue Engineering

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Silk/silica chimeric proteins are studied as new biomimetic nanocomposites for bone repairs and tissue engineering. Spider dragline silk consensus repeats represent a protein self assembling domain that form highly stable (beta-sheet) secondary structures with outstanding mechanical properties that rival the strongest synthetic fibers. The silica forming domain derived from the silicatein protein of a diatom offers fine control over the formation of silica nanostructures with tunable morphologies. The process to generate these protein fusions assures tailored nanocomposite materials that can be generated with useful functional performance towards new bone formation. These fusion proteins provide a novel approach to nanoscale materials assembly leading to well-organized composite structures with control of organic-inorganic interfaces to optimize material features. The impact of modifications on the molecular level in the organic phase (silk repeats), as well as different chemistry in silica precipitating domains (chemically designed peptides), are assessed for their influence on material properties such as morphology, structure, and mechanics. Outcomes are also assessed in terms of impact on bone regeneration. The studies to date indicate successful formation of such nanocomposites with remarkable mechanical properties, different morphologies, and biocompatibility. In a related approach we examine blended protein biomaterials of silk fibroin with different sizes of silica nanoparticles towards osteogenic differentiation of human mesenchymal stem cells (hMSC's). In vitro studies are utilized to monitor the interaction of the cells with the bioengineered nanocomposites towards osteogenic outcomes.