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*

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

Efraim Feinstein[1], Eben Alsberg[2], Donald Ingber[3], Mara Prentiss[4].
[1]Dept of Physics and Program in Biophysics, Harvard University, Cambridge, MA, USA, [2]Dept of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA, [3]Harvard Institute for Biologically Inspired Engineering, Harvard School of Engineering of Applied Sciences, Harvard Medical School, and Childrenï¿½ s Hospital., Boston, MA, USA, [4]Dept of Physics, Harvard University, Cambridge, MA, USA.

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

Allen Hirsh, Latchezar I. Tsonev.
Cryobiophysica, Inc., Rockville, MD, USA.

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.

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Development Of Novel Biomimetic Membrane Designs For Separation And Biosensor Applications

Jesper S. Hansen[1,2], Mark Perry[2], Jörg Vogel[1], Thomas Vissing[2], Sania Ibragimova[3], Christian R. Hansen[4], Pierre-Yves Bollinger[2], Kamilla J. Pszon[1], Oliver Geschke[1], Jenny Emnéus[1], Jenny Emnéus[1], Claus H. Nielsen[3,2].
[1]DTU Nanotech, Technical University of Denmark, Lyngby, Denmark, [2]Aquaporin A/S, Lyngby, Denmark, [3]DTU Physics, Technical University of Denmark, Lyngby, Denmark, [4]Science Center, Copenhagen University, Copenhagen, Denmark.

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.

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Silk/silica Nanocomposites As Novel Biomaterials For Tissue Engineering

Aneta J. Mieszawska[1], David Kaplan[1], Carole C. Perry[2].
[1]Tufts University, Medford, MA, USA, [2]Nottingham Trent University, Nottingham, United Kingdom.

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.