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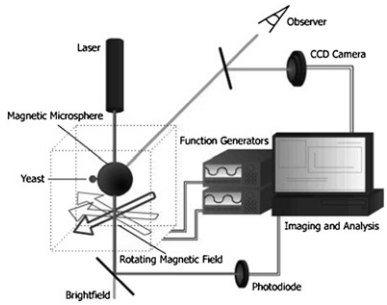
We are exploring the use extracellular fluorescent indicators to measure analytes in the microdomains between adjacent cells in tissues. Our primary initial goal is to measure changes in $H^+$ activity in the intercellular space of xenograft tumor models before and after drug treatment. It is thought that acidification in the core of tumors protects these cancers from the primarily, weak base, chemotherapeutic drugs. We are comparing the use of functionalized nanoparticles and expressible indicators as putative extracellular sensors. Functionalized, fluorescein-labeled nanoparticles were constructed that enable the particles to adhere to the surface of schwannoma cells. However, preliminary work with the nanoparticles showed toxic side effects, at least over a period of days. We have also been exploring the use of expressible membrane bound $H^+$ indicators, glycosylphosphatidylinositol (GPI)-anchored GFP mutants. A problem with these indicators is that they localize to the plasma membrane and also the entire exocytotic pathway. *In vitro* testing of the indicators was accomplished by aggregating single cells into clusters (artificial tumors) using dielectrophoresis (DEP) and agarose molds. Preliminary results indicated that nanoparticles could be trapped within artificial tumors using DEP as long as electric fields are applied. Long term (7 days) intercellular pH sensing was achieved *in vitro* using GPI-GFP expressing schwannoma cells in an artificial tumor crafted with an agarose mold. While we are currently exploring the use of sensors to measure changes in pH in the intercellular space, we are also investigating the development and testing of other sensors to detect analytes such as ATP, $Cl^-$, $Na^+$, and $K^+$. This will provide valuable information regarding cellular defenses against chemotherapeutic drugs and mechanisms of therapeutic drug action.
*This project was funded by the NIH:NCRR as a national research resource [P41 RR001395]*

**3264-Pos Board B311**
**Using Automated Cell Tracking Software to Quantifying Durokinesis and Durotaxis in Real Time**
**Matthew L. Walker**, David House, Margrit Betke, Joyce Y. Wong.
Boston University, Boston, MA, USA.
In vivo, there is an intimate connection between certain cellular processes and the physical nature of the surrounding environment. Specifically, it has been theorized that changes in the physical properties of the extra-cellular environment within the vasculature influence cellular migration which can influence such processes as angiogenesis and occlusive vascular disease.
In order to observe and quantify the compliance directed migration of both vascular smooth muscle cells and fibroblasts, we employed both polyacrylamide substrates, in which the tensile modulus could be tuned to specific values, and novel computer imaging software which automatically tracked cellular movement in less then ideal optical imaging conditions.
Although both durotaxis and durokinesis have been previously observed in large population studies, our application of computer vision software allowed for a high throughput analysis of individual cells in real time. This method not only standardized the data collection but also enabled us to observe and quantify changes in speed, angular deviation, acceleration and deceleration within a single cell's migration track as a function of substrate stiffness and in the presences of a compliance gradient. This detailed analysis will serve to refine our understanding of cells respond to the physical stimuli presented in the environment.

**3265-Pos Board B312**
**Asynchronous Rotation as a Rapid and Sensitive Technique for Quantifying Cell Growth Dynamics**
**Daniel W. Youngstrom**, Brandon McNaughton, Päivö Kinnunen, Raoul Kopelman.
University of Michigan, Ann Arbor, MI, USA.
The ability to monitor physical parameters of cell growth dynamics on an increasingly sensitive scale is of great interest. Nonlinear rotation of cell-coated magnetic microspheres is an exciting new technique for rapid cell detection and measurement. Previous explorations of this approach primarily involved bacteria and other prokaryotes, but new methods demonstrate it is possible to extend this model to the world of eukaryotes, specifically simple yeasts. In this experiment, *Saccharomyces cerevisiae* cells were coated in biotin and covalently linked to a streptavidin-coated magnetic microsphere. With their cell membranes bound to the sphere, the unit was rotated asynchronously in a magnetic field. As the cells grow, the viscous drag experienced by the cell-bearing microsphere increases, counteracting the magnetic driving force, yielding a steady overall increase in rotational period. The rotation rate is actively monitored by specialized computer software using the voltage output of a laser and photodiode.




Current data reinforces the success of dynamic asynchronous rotation as a valid means for rapid and sensitive growth detection. In the future it is believed this technique may be further extended to the study of increasingly complex organisms, including mammalian cells.

**3266-Pos Board B313**
**Detection Of Drinks Contamination Using Optical Refractometry Technique (ORT)**
**Christopher E. Bassey**, Cynthia A. Siguenza.
Azusa Pacific University, Azusa, CA, USA.
Optical Refractometry Technique (ORT) has been used to characterize unknown substances, check the concentration of known substances, and also determine the sugar content of a given liquid. It involves the determination of a medium's refractive index, which is a measure of the speed of light in the medium. The technique is based on the principle that the speed of light in a given medium is a reflection of its absorption and emission characteristics. The speed of light also depends on the physical state, composition, and molecular structure of the medium. By comparing the optical properties of pure drinks with those of drinks tainted with foreign agents, the level of contamination can be detected. This work examines the feasibility of using ORT to detect the contamination Gatorade drink with antifreeze, which has already led to a number of deaths. The results will enhance the development of instrumentation and methodology for continuous monitoring and detection of possible contamination.

**3267-Pos Board B314**
**Assessment of Cytotoxicity by Analysis of Impedance Fluctuations**
**Jun-Chih Lo**, Daniel Opp, Chun-Min Lo.
University of South Florida, Tampa, FL, USA.
Electric cell-substrate impedance sensing (ECIS) has been used to monitor cell behavior in tissue culture and has proven to be very sensitive to cell morphological changes and cell motility. In this method, cells are cultured on small gold electrodes carrying weak AC signals. The impedance of these electrodes changes dramatically when cells attach and spread on their surface, because the cell membranes restrict the current flow. In addition, cell motion may reveal itself as a fluctuation in the measured impedance, which is always associated with living cells and persists even when the cells grow into a confluent layer. The impedance fluctuation is attributed to incessant changes in the size of the cell-substrate space as cells persistently rearrange their cell-substrate adhesion sites. The magnitude of this sort of vertical motion detected by ECIS is of the order of nanometers and referred to as micromotion. Here, we applied ECIS to evaluate dose-dependent responses of NIH 3T3 cells exposed to cytochalasin B, cadmium chloride, and H-7 dihydrochloride, a protein kinase C inhibitor. To detect the alternation of cell micromotion in response to cytotoxic challenge, time-series impedance fluctuations of cell-covered electrodes were monitored and the values of power spectrum, variance, and variance of the increment were calculated to verify the difference. While a dose-dependent relationship for each chemical was generally observed from the overall resistance of the cell monolayer, the analysis of impedance fluctuations distinguished cytochalasin B, cadmium chloride, and H-7 dihydrochloride levels as low as 0.1, 10, and 1 micromole respectively. The analytical methods used in this study can serve as a model approach for ECIS and other electrochemical impedance biosensors to investigate various aspects of cellular responses to toxins in general.

**3268-Pos Board B315**
**Towards In Silico Bioprinting**
**Adrian Neagu**[1,2], Elijah Flenner[1,3], Bogdan Barz[1], Cyrille Norotte[1], Francoise Marga[1], Karoly Jakab[4], Ioan Kosztin[1], Gabor Forgacs[1].
[1]University of Missouri-Columbia, Columbia, MO, USA, [2]Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, [3]Colorado State University, Fort Collins, CO, USA, [4]University of Virginia, Charlottesville, VA, USA.
Bioprinting is a computer-controlled procedure for building three-dimensional tissue constructs via layer-by-layer delivery of cells and supportive hydrogels. To describe the post-printing self-assembly of multicellular structures, we performed computer simulations that incorporate a basic principle of developmental biology, the differential adhesion hypothesis (DAH). DAH states that cell motility combined with differences in the adhesive properties of different cell types yields tissue conformations with the largest number of strong bonds

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**

**Gabor Forgacs**, Francoise Marga, Carina Poltera, Cyrille Norotte.
University of Missouri, Columbia, MO, USA.

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**

**Sushanth Gudlur**, Matt Warner, Yasuaki Hiromasa, Takeo Iwamoto, John M. Tomich.
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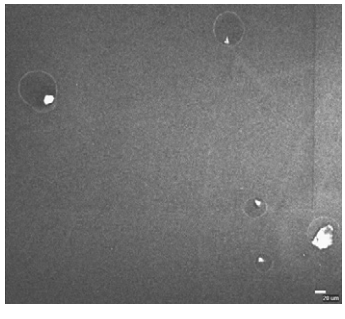
Self-Assembling peptide nanovesicles are attractive candidates for drug delivery. Here we report the characterization of specific amphiphilic peptides (up to 23-amino acids in length) that undergo supramolecular assembly to form both mono- and hetero-assemblies that form nanomicelles and larger vesicles (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**

**Francoise S. Marga**, Bradley Hubbard, Tom McEwan, Stephen Colbert, Gabor Forgacs.
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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**

**Leslie R. Farris**, Wenhui Wang, A. Clarizia Lisa-Jo, Xingwei Wang, Melisenda J. McDonald.
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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