

heparin. A set of methods was applied: Surface Plasmon Resonance (SPR) was chosen to continuously monitor the self-assembly process of physi-sorption of subsequent PE layers and to report about deposition efficiency, dynamics and stability of the PEM film. Atomic force microscopy (AFM) was used to visualize and characterize the surface topology obtained after subsequent steps of the layer-by-layer deposition. The multi-layer structure of the composite film and its hydration were studied with neutron reflectometry. The knowledge gathered so far on this system is now being applied for the deposition of lipid bilayers and other biomimetic systems for subsequent biophysical studies.

3151-Pos

Analysis of Lipid Compositional Changes During Alcoholic Fermentation in Industrial Yeast Strains with Varying Ethanol Tolerance

Clark M. Henderson, David E. Block, Marjorie L. Longo.

University of California, Davis, Davis, CA, USA.

In model lipid bilayers, ethanol is known to alter the mechanical and thermodynamic properties of the membrane. The extent to which alcohol affects these properties depends upon the lipid composition of the bilayer. Our group has demonstrated that increased ergosterol levels in model membrane systems mitigate the membrane thinning effect of ethanol - a phenomenon known as interdigitation. Perturbations to the yeast biomembrane due to increasing ethanol levels has been implicated in reduced sugar utilization and cell viability in *Saccharomyces* sp. However, variations in fermentation conditions and analytical methods have not yielded a comprehensive picture of how yeast biomembranes adapt to increasing levels of ethanol. In this work, we analyzed the partial lipidome of 30 industrial yeast strains at different stages of fermentation using high-resolution mass spectrometry. Quantification of selected lipid species was performed using high performance liquid chromatography coupled online to quadrupole ion-trap mass spectrometry. Multivariate statistical analysis of the quantitative data was performed to determine any correlations between changes in lipid composition and ethanol tolerance in the different yeast strains. Information regarding how yeast biomembranes adapt to greater ethanol concentrations will be used to construct biophysical models to analyze the complex physical properties of lipid biomembranes in an alcohol milieu.

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Automated Lipid Bilayer Formation using a PDMS Gasket

You-Hyo Baek¹, Joongjin Park¹, Jason L. Poulos², Jacob J. Schmidt²,

Tae-Joon Jeon¹.

¹Inha University, Incheon, Republic of Korea, ²University of California, Los Angeles, Los Angeles, CA, USA.

Planar lipid bilayer membranes (BLMs) play important roles in studying ion channels, as well as in potential applications for drug discovery. Despite the importance of many practical applications using BLMs, membrane formation is still based on conventional methods invented by Montal and Mueller in 1960s. Although membranes can be simply reconstituted using the conventional technique, membranes should be created where experiments are conducted due to their mechanical instability. In our recent work a membrane formation technique using high melting temperature solvent mixture was devised by Jeon, et al. (Lab on a Chip, 8(9): 1742 (2008)) Briefly, 2:8 mixture of n-decane and hexadecane was spread over a small aperture and froze before its spontaneous self-assembly process to a bilayer membrane. Since the membrane precursor can be created in a central facility and shipped to any place, it can be transported to any place and thawed when a membrane is needed, widening the usability of artificially created lipid bilayer membranes. Nevertheless the main drawback of the conventional technique was not completely ameliorated in this work due to the membrane support. Since a membrane precursor was deposited in a small aperture on a plastic sheet, the membrane formation process by self-assembly was unchanged, resulting in variations in membrane formation time with a range of ~30 minutes to 24 hours. In our work a PDMS gasket was used to support membrane structure in place of a plastic sheet. Since organic solvent can be extracted into the PDMS gasket, a lipid bilayer membrane can be formed within ~30 minutes in a controlled manner. Ion channels incorporated into a membrane formed in a PDMS gasket functioned as in a conventional membrane. Furthermore, we will show the broad applicability of our membrane formation technique.

3153-Pos

Fabricating a New Stabilized Lipid-Based Platform for Handling and Presenting GPCRs

Yasaman Dayani, Noah Malmstadt.

Mork Family Department of Chemical Engineering and Materials Science, University of Southern California, Los Angeles, CA, USA.

G protein-coupled receptors (GPCRs) are members of large family of signaling molecules. They have a key function in neuronal biology and play a critical role in transmitting extracellular signals in eukaryotic cells. GPCRs include receptors for many neurotransmitters like serotonin. The serotonin receptor 5HT_{1A} has an important role in mental disorders like anxiety, and is the target of some anti anxiety drugs. Here we constructed a novel biomimetic lipid membrane-based platform to be used for screening molecules that interact with GPCRs. Since fragility and short life time limit the applicability of liposomes that are normally used to present membrane proteins, we have developed a platform based on nanoscale liposomes with enhanced longevity and stability. We developed two approaches to make stable liposomes: liposomes containing a UV-initiated poly(ethylene glycol) (PEG) hydrogel and conjugated hydrogel liposomes made from lipids covalently anchored to the hydrogel network. Stability of nanoscale liposomes was confirmed by addition of high concentration of sodium dodecyl sulfate (SDS) to the liposome suspension. The liposome/detergent micelle mixture was passed over a size exclusion chromatography (SEC) column, separating intact liposomes from micelles. Using dynamic light scattering (DLS), we could confirm the existence of intact 160 nm liposomes. This result was in good agreement with the SEC result for polymerized liposomes without detergent. Since our goal is making GPCR-bearing liposomes, serotonin receptor 5HT_{1A} was incorporated into liposomes using a detergent-mediated method. GPCR incorporation was confirmed by binding labeled HTR_{1A} antibody to liposomes containing biotinylated lipids and subsequently separating them from bulk with streptavidin-coated magnetic beads. We are currently undertaking further studies on antibody-liposome binding by fluorescence anisotropy to validate our approach.

3154-Pos

In Vitro Enhancement of Collagen Deposition in Fully Biological Bioprinted Constructs

Francoise S. Marga, Troy Young, Gabor Forgacs.

University of Missouri-Columbia, Columbia, MO, USA.

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. As the cells grow and deposit their own extracellular matrix (ECM), the scaffold degrades slowly. After implantation, remnants of the scaffold can trigger chronic inflammation and create mechanically weak zones by interfering with extracellular matrix assembly. Over the last few years, we developed an alternative, scaffold-free method. Our technique exploits well-established developmental processes (such as tissue fusion, spreading and cell sorting). 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 tubular constructs. Structure formation takes place by the post-printing fusion of the discrete units and maturation in bioreactor. The slow buildup of cell-produced ECM needed for adequate mechanical strength before implantation, however remains an issue. Collagen deposition is hindered *in vitro* due to the slow conversion of de novo synthesized procollagen to collagen before its secretion.

Here we explore different strategies to enhance collagen deposition in the bio-ink units and the post-fusion construct. Collagen deposition could be observed throughout spheroids obtained by seeding human aortic smooth muscle cells in 96 well plates and culturing them in presence of ascorbic acid. However the process still took 3 weeks. Boost in collagen production by the bio-ink units was observed when negatively charged dextran sulfate (DxS, 500 kDa) was added to the medium. After high content bio-ink units fuse into a tube, ECM reorganization is expected under mechanical stimulation in the bioreactor. In conclusion, DxS could fasten the production of ECM in tissue engineering applications.

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