through a synthetic bilayer lipid membrane (BLM) are measured to obtain a rapid and sensitive signature of BLM disruption. In this study, we describe application of electrophysiology methods to investigate molecular interactions between a variety of ENM and BLM. To explore the effect of ENM surface properties on ENM-BLM interactions, BLM were challenged with custom-fabricated polypropargyl glycolide nanoparticles having a variety of surface functional groups. Polyethylene glycol functionalized ENM triggered ionic current spikes having a duration of milliseconds, while carboxyl-functionalized ENM generated both rapid current spikes and extended integral conductance. Ion selectivity of the ENM-generated conductance was investigated by measuring reversal potential of transmembrane currents at various applied potentials in the presence of a 10:1 KCl gradient. Reversal potentials for different types of ENM varied from 0 mV, indicating no ion preference, to 37 mV, indicating preference for K^+. To explore the effect of BLM composition on ENM-BLM interactions, two BLM compositions were tested: pure DOPC, and a 3:1 ratio of POPC:POPE. For a variety of ENM, the DOPC BLM consistently exhibited ion currents at lower concentrations and lower transmembrane voltages than did the POPC:POPE BLM. These results demonstrate the utility and versatility of electrophysiology methods to characterize molecular interactions between ENM and BLM.

2963-Pos Board B733

Poregenic - Patch on Chip System for Adherent Cellular Networks

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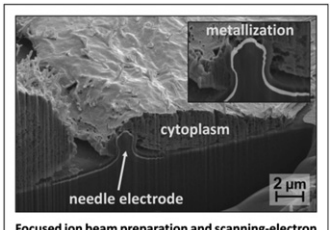
Automated patch-clamp setups are applied to investigate dose response relationships and target kinetics in the development of new pharmaceutical agents. Currently, automated systems are limited to investigations of suspended single cells. We pursue the development of assays for detecting the membrane properties in adherent networks because the majority of cells in humans grow adherently. In a first step, we developed PoreGenic®, a novel patch-clamp system for cells growing on a sensor chip with micro-structured needle electrodes arranged in an 8×8 multi-electrode array with a pitch of 100 µm. PoreGenic® allows for the electrical cell manipulation as well as for extra- and intracellular potential measurements. Four types of needle electrodes of different shapes and materials were tested with heights of less than 10 µm. For intracellular detection, electroporation pulses were applied to form membrane pores for the introduction of the electrodes into the cytoplasm. Fluorescence and scanning-electron microscopy in combination with focused ion beam preparation were used to characterize the success of electroporation. In a number of experiments, we could access the cytoplasm and detect intracellular potentials. Our current system features 16 hollow needle structures with fluidic connections for patch-clamp experiments.



Focused ion beam preparation and scanning-electron micrograph of an electroporated L929 tumor cell. *Preparation and image: Fraunhofer IWM Halle, Germany. Chip fabrication: IMTEK, University of Freiburg, Germany.*

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Biological Activity of GABA Neurotransmitter Conjugated with Gold Nanoparticles in Xenopus Laevis Oocytes Transfected with GABA Rho 1 Receptor

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Gold nanoparticles are of great interest to the scientific community owing to their potential applications in biology and medicine, earned by their extraordinary optical and chemical properties; counting their excellent biocompatibility, colloidal stability and suitable surface properties for conjugation with biomaterials. In this study, GABA, a classic neurotransmitter was conjugated with gold nanoparticles (Au@GABA). The gold nanoconjugates were synthesized by forming a covalent bond between gold nanoparticles and GABA. Au@GABA nanoconjugates showed excellent optical features and superior stability under physiological conditions. Results from UV-Vis, FTIR and Raman spectroscopies proved the optimum optical properties and chemical composition of the nanoconjugates. The surface charge was characterized by gel electrophoresis and the morphological properties by scanning electron microscopy. Bare gold nanoparticles and nanoconjugates were tested by electrophysiological recording in oocytes of Xenopus laevis expressing GABA rho 1 receptor.

2965-Pos Board B735

Localization of Liquid-Ordered/Liquid-Crystalline Phase Separation at a Lipid Bilayer Suspended Over Microwells

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We investigated the localization of liquid-ordered (L_o) and liquid-crystalline (L_α) phase domains in a lipid bilayer suspended over microwells on a silicon substrate. It has been suggested that membrane trafficking and signal transduction in cellular processes are driven by functional rafts. The localization of L_o and L_α phase separation as a model of a lipid raft plays an important role regarding the arrangement and function of membrane proteins. When we fabricate a nanobiodevice that works with membrane proteins, controlling the function of proteins is a key technique. A microwell array with a sealing lipid bilayer is a promising platform, and we believe that our findings reported in this manuscript will contribute to the fabrication of nanobiodevices.
Suspended lipid bilayers were formed by rupturing giant unilamellar vesicles (GUVs) on the substrate. The GUVs were prepared from ternary mixtures of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and cholesterol. We found that the L_α phase is preferable at the suspended membrane because of its curvature. Phase separation and localization are governed by the stiffness and line tension of the membrane. Lateral diffusion at the molecular level occurs quickly, although domain movement is restrained at the supported membrane by the interaction with the substrate. Unsaturated lipid is condensed at the suspended membrane, and saturated lipid and cholesterol are excluded. Additional phase transition and domain rearrangement occurs. The localization of L_o/L_α phase separation can be controlled by the microwell array on the substrate.

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Nanostraws for Direct Fluidic Intracellular Access

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Delivery of small molecules, proteins, and genetic material across the cell membrane barrier and into the cytosol is a critical step for molecular biology and cell reprogramming techniques, yet efficient, non-disruptive delivery is still often a rate-limiting step. We demonstrate cell culture platforms of fluidic ''nanostraws'' that pierce the cell membrane, providing a permanent pipeline to the inside of the cell for direct material delivery. Conventional polymeric track-etch cell culture membranes are alumina coated and etched to produce fields of nanostraws with controllable diameter, thickness and height. Each nanostraw is fluidically connected to the bottom of the polymer membrane, allowing chemicals located under the cell culture area to diffuse through the nanostraws and directly into the cells. Small molecules, ions, and green fluorescent-protein-encoding DNA plasmids were successfully transported into the cytosol with efficiencies up to 70%. Depending on the underlying fluidic chamber design, species could be delivered uniformly over large areas of ~10^6 cells, or selectively within a narrow band. Although not all nanostraws penetrated the cell membrane, those that did remained open over extended periods, enabling sequential chemical delivery and modulation. Remarkably, nanostraw penetration does not appear to perturb the cells, as both live/dead assays and mRNA gene chip analysis show no statistical differences with control populations. These platforms open the way for simple, reproducible delivery of a wide variety of species into cells without endocytosis.

2967-Pos Board B737

An Integrated Cell Lysis Chip

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This paper describes an integrated lysis chip that utilize syringe pump for the detection of the Beta-amyloid protein in cells or tissues. An integrated lysis chip that has many advantages such as easy control, simply and novel design, cost and time effective. The integrated lysis chip that was fabricated in poly (dimethylsiloxane) (PDMS) form masters prepared by soft lithography using SU-8 3035 photoresist. The integrated lysis chip has two main components: the lysis channel with blades containing an input port and connects port to filter system channel, and the filter system channel which has an output port and reservoir chamber. The filter system channel was constructed using multi layers of commercial filters. The integrated lysis chip is used here to lysis, concentration and precipitation. The chip was successfully evaluated using NIH3T3 cells. The