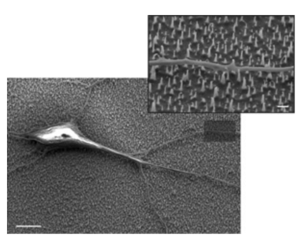
Micro and Nanotechnology; Nanopores III

3280-Pos Board B385
A Multiplexed Electrochemical Microelectrode Array for High-Throughput Measurement of Quantal Exocytosis
Jia Yao, Kevin D. Gillis.
Electrochemical microelectrodes are commonly used to detect spikes of amperometric current that correspond to exocytosis of oxidizable transmitter from individual vesicles, i.e., quantal exocytosis. We are developing transparent multi- electrochemical electrode arrays on microchips in order to automate measurement of quantal exocytosis. Whereas patterning hundreds of electrodes in a small area is straightforward and cost-effective using photolithography, easily making connections between hundreds of electrodes and external amplifiers remains a bottleneck. Here we report a simple multiplexing approach using multiple fluidic compartments that can reduce the number of external connections by ~100-fold. In this approach the set of electrodes in every fluidic compartment are wired in parallel and connected to external amplifiers. Cell recordings are made from the set of electrodes in one fluidic compartment at a time. The fluidic compartment to be used is addressed by loading it with cells in a physiological electrolyte solution and connecting it to the ground / reference electrode. Measurements demonstrate that this approach attains current noise levels as low as that obtained with individual electrodes. However, if electrolyte solution is in more than one fluidic compartment the noise increases proportionately. An equivalent circuit model has been developed to quantify this noise as resulting from the thermal noise of the electrode / electrolyte junction. The new device will enable high-throughput studies of quantal exocytosis that can be combined with fluorescence microscopy. Supported by NIH R01 NS048826.

3281-Pos Board B386
Nano-Scale Surface Topology Improves Neuronal Development in Culture
Ghislain Bugnicourt, Jacques Brocard, Mariano Bisbal, Nora Collomb, Annie Schweitzer, Catherine Villard.
Neurons may be grown on opaque silicon surfaces covered with poly-L-lysine, the same way it is usually carried out on glass coverslips. Neuronal development may also be improved by structuring silicon surfaces with reactive ion etching. Here, we produced silicon samples by a classical photolithography process with an alternation of 1mm-wide bands, presenting atomically flat or rough topologies.
Hippocampal neurons from E18.5 mice, grown over these samples, developed faster on rough bands: they differentiated an axon more readily after 2 days in culture (75% neurons display an axon vs 60% on flat surfaces) and their total neuritic length was 50% larger whereas they developed 20% less neurites. Moreover, neurons with neurites in both areas preferentially differentiated an axon onto the rough surface (76% polarized neurons).
Finally, we characterized the topology of rough surfaces using Scanning Electron Microscopy. We observed 1μm-high silicon nano-peaks randomly distributed on the surface. Interestingly, our observations indicated that neurites grew on top of the nano-peaks with a typical distance between adhesion points of about 600nm. Neuron on rough silicon surface (bar, 5 μm), and zoom over a neurite (dark rectangle and inset; bar, 500 nm).



3282-Pos Board B387
Adaptive Mechanically Controlled Lubrication Mechanism found in Articular Joints
George W. Greene.
Articular cartilage is a highly efficacious water-based tribological system exhibiting low friction and wear over a lifetime despite its biological isolation and slow ability to regenerate. Hyaluronic acid (HA) is abundant in cartilage and synovial fluid and widely thought to play a principle role in joint lubrication and proper joint function although this role remains unclear. HA is also known to complex readily with the surface active glycoprotein lubricin (LUB) to form a cross-linked network and is also believed by some to be important to the lubrication and wear prevention mechanism of joints, although again it's role is unclear, especially the synergy in the actions of LUB and HA. Friction experiments on porcine cartilage using the surface forces apparatus, and enzymatic digestion, reveal an ''adaptive'' role for an HA-LUB complex whereby, under compression, nominally free HA diffusing out of the cartilage becomes mechanically, i.e., physically, trapped at the interface by the increasingly constricted collagen pore network. The mechanically trapped HA-LUB complex now acts as an effective (chemically bound) 'boundary lubricant' - reducing the friction force slightly but, more importantly, eliminating wear damage to the rubbing/shearing surfaces.

3283-Pos Board B388
Towards the World Smallest Chemical Reactors: On-Demand Generation and Fusion of Femtoliter Aqueous Droplets
Seung-Yong Jung, C. Patrick Collier, Scott Retterer.
Water in oil droplets has been an important research topic because of the possibility of small-volume biochemical reaction vessels in which the reaction time and chemical concentration can be precisely controlled. Here, a new method for producing *femtoliter-scale* (10^{-17} L) aqueous droplets on-demand has been developed based on pressure driven formation of droplets at the intersection of microchannels. Different aqueous solutions in two apposed microchannels with 1 micrometer in width were forced into an oil-filled microchannel to form two aqueous droplets, which then collided with each other to start biochemical reactions by diffusional/convectional mixing. These monodisperse micro-reactors can enable the characterization of catalytic or biochemical reaction dynamics in confined environments, as well as the development of reaction vessels for highly localized sampling and dosing.

3284-Pos Board B389
A Combined Surface Chemistry / Microwell Approach for Trapping Single Cells on Electrochemical Microelectrodes for Measurement of Quantal Exocytosis
Xin (Alice) Liu, Syed Barizuddin, Wonchul Shin, Cherian J. Mathai, Shubhra Gangopadhyay, Kevin D. Gillis.
Electrochemical microelectrodes are commonly used to detect spikes of amperometric current that correspond to exocytosis of oxidizable transmitter from individual vesicles, i.e., quantal exocytosis. We are developing transparent multi- electrochemical electrode arrays on microchips in order to automate measurement of quantal exocytosis. Here we report the development of an improved device to target individual cells to each microelectrode in an array. Efficient targeting (~75%) was achieved using cell-sized micro-well traps fabricated in SU-8 photoresist together with patterning of poly (L-lysine) in register with electrodes to promote cell adhesion. The surface between electrodes was made resistant to cell adhesion using poly (ethylene glycol) grafted to a poly (dopamine) adhesive in order to facilitate movement of cells to electrode microwells. We demonstrated the activity of the electrochemical microelectrodes using the test analyte ferricyanide and perform recordings of quantal exocytosis from bovine adrenal chromaffin cells on the devices. Dozens of cell recordings on a single device illustrated the consistency of recordings and multiple recordings from the same electrode demonstrated that the device could be cleaned and re-used without degradation of performance. The novel device will enable high-throughput studies of quantal exocytosis and may also have applications in rapidly screening drugs or toxins for effects on exocytosis. (Supported by NIH R01 NS048826.)

3285-Pos Board B390
Micro- and Nanoparticle Translocation through a Solid-State Membrane Pore Thinner than their Diameters
Ken Healy, Matthew Davenport, Sonia E. Letant, Zuzanna S. Siwy.
Micron-sized particles have been detected by the resistive-pulse or Coulter counting technique since the 1950's (Coulter, US Pat. 2656508) with 90 nm particle detection reported in 1970 (DeBlois and Bean, Rev. Sci. Inst. 41, 909). Due to the challenges of fabricating submicron pores in those times, the pores used were all several microns in length. Nanofabrication has advanced significantly since then, and in this poster we examine the interesting case of particles translocating through a membrane pore thinner than those particles.
With micron-long, high aspect ratio pores, a spherical particle will be entirely contained within the pore for the majority of the time it translocates. Thus, all but the beginning and end of the observed signal is due to the entire particle. In addition, pore resistance dominates over access resistance. In contrast, with low-aspect ratio pores thinner than the particle, the signal reflects the interactions of the particle with both the access resistance volumes and the pore volume. The signal is a complex combination of these interactions, that changes as the particle moves through the pore. Another advantage of shorter pores is that identical particles passing through longer pores give smaller modulations in current, because the proportional change in resistance due to the particle is smaller.
We use a focused ion beam to drill nanopores tens to hundreds of nanometers in diameter in silicon nitride membranes, with thicknesses in the same range. Polystyrene microspheres are driven through these pores by an applied electric field. As they pass through, they modulate the flux of ions through the pore, and thus the current flowing between the electrodes. We present experimental and