photorespiration where PSI substitutes cytochrome oxidase and PSII temperature sensitive mutation. We suggest that for the first time our system will be able to separate oxygen evolution and efficient hydrogen production.

3128-Pos

Characterization of New and Improved Fluorescent Proteins and their Applications

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Fluorescent proteins (FPs) have become ubiquitous tools in biological and biomedical research. Since the cloning and exogenous expression of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, researchers have sought new variants of this and other FPs, with properties well-suited for particular imaging applications. Of special interest are FPs with different excitation and emission wavelengths, with brighter fluorescence, monomeric, and that mature rapidly at 37°C. We have examined the properties of several novel FPs isolated from fluorescent marine organisms, which were collected from the Great Barrier Reef in Australia and Belizean Barrier Reefs. All the proteins were cloned and expressed in *E. coli* and their properties were characterized. One FP, 28bc2, showed very promising characteristics having high brightness and a weak propensity to form dimers. We have modeled the structure of 28bc2 and designed mutations at the putative dimerization interface to decrease the potential to dimerize. We have further characterized the spectroscopic properties of 28bc2 wild type (wt) and the mutants and compared their properties with those of EGFP, a widely used variant of GFP. The 28bc2 wt and mutants FP are at least 2-fold brighter than EGFP and show similar pH stability profiles to that of EGFP. The photostability of 28bc2 wt and mutants is less than that of EGFP, though for some applications this is not critical. We have shown the advantages of brightness of 28bc2 mutants in one-step detection application in Western blotting and their usefulness in *in vivo* labeling demonstrated by RNA microinjection assays in zebrafish.

3129-Pos

Optimizing Functionality of Ion Channel Biosensing using Stochastic Resonance

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Stochastic resonance refers to the increased sensitivity of a system when a finite level of noise is applied to the system. This counter-intuitive concept is evidenced by a maximum in the signal-to-noise ratio with respect to applied noise level. We have applied this technique to a system of alamethicin ion channels incorporated in a planar lipid bilayer. When used as a molecular biosensor, an enhancement of the signal-to-noise ratio of such a system improves the sensor's limit of detection. Thus, by adding noise to the biosensor, we can maximize its sensitivity. We also show how this technique can be used to design an inherently optimal molecular biosensor. By varying the *lipid membrane area*, the alamethicin concentration, and applied voltage in each system, we control the level of noise internal to the system. Then, by noting the level of *external* noise that induces stochastic resonance, we inferred the level of *internal* noise that each variable introduces to the system. In doing so, we found that microphonic noise, which is introduced by the lipid membrane, most significantly influences the signature of stochastic resonance. Thus, we have shown that by tuning the membrane area to induce an optimal level of microphonic noise, one can design a molecular biosensor that inherently induces stochastic resonance.

3130-Pos

Simultaneous Recordings of Ligand-Gated Ion Channels using a 384 Planar Patch Clamp Substrate

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We have developed an apparatus that allows the simultaneous measurement of ligand-gated ion channels (LGICs) at 384 separate recording sites in parallel prior to, during and after ligand addition. Since the development of planar patch clamp recording techniques in 2002 the number of parallel recordings that could be done on LGICs has been limited to 48. Our apparatus measures cell membrane currents using the perforated patch clamp techniques on a polyimide substrate. Currents are measured using a single hole at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Finkel et al. 2006). PPC averages the membrane currents in the 64 cells at each recording site by measuring the ensemble current through all 64 cells using a single pair of electrodes. PPC increases the success rates by mitigating biological variability caused largely by cells not expressing the channel of interest. We present here the consistent ability to measure cell membrane currents simultaneously from all 384 sites. Data presented include LGIC recordings of GABA chloride channels, acid sensing ion channels (ASIC), and nicotinic acetylcholine (α1 nACh) receptors. In addition to the LGIC data presented we also present recordings of voltage-gated ion channels (VGICs) including Na_V, K_V and hERG channels. Pharmacological blockade of ion channel activity is also presented to validate the use of this apparatus for screening ion channel targets in a drug discovery setting.

Ref: Finkel, A. et al. (2006). J Biomol Screen **11**(5): 488-96.

3131-Pos

Planar Patch-Clamp Electrodes for Single Cell and Neural Network Studies

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The traditional glass pipette patch-clamp technique has contributed greatly to fundamental and pharmacological ion channel studies. The success of this serial technique has driven an effort to create wafer-based patch-clamp platforms using materials with inferior dielectric properties than glass and/or using exotic processing techniques to avoid the difficulties inherent to parallel processing of glass. We have developed a material processing scheme that generates ultrasmooth, high aspect ratio pores in fused quartz wafers. These devices are demonstrated here to be superior planar patch-clamp electrodes achieving gigaohm seals in nearly 80% of trials with a mammalian cell line, with the majority of seals over 10 GΩ and as high as 80 GΩ, competing with the best pipette-based patch-clamp measurements. Our method, amenable to batch fabrication technologies, will enable the acquisition of low noise, ion channel measurements in high throughput.

We are currently merging the abovementioned devices with voltage sensitive dye (VSD) imaging and multi-electrode array (MEA) recordings in order to study multisensory integration in the medicinal leech. Initially, the planar pores will function to provide precise placement of neurons in the leech ganglion over the MEA's. The excellent spatial resolution of the VSD's combined with the temporal resolution of MEA's will provide much information of all the neurons that respond to visual stimuli in the ganglion. Further studies may employ the planar pores as intracellular electrodes, allowing voltage control and intracellular recordings of individual neurons in the ganglion.

3132-Pos

A Two-Channel Patch-Clamp System on a Chip

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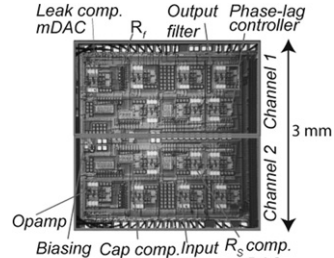
High-throughput patch-clamp systems require a large number of amplifiers in a small area. Towards a solution to this problem, we have implemented a two-channel patch-clamp system on a chip in a 3 x 3 mm area using silicon-on-sapphire (SOS) technology. The system is capable of compensating series resistances and the pipette capacitances up to 100 MΩ and 10 pF respectively. The system is able to compensate 100 % of the series resistance using phase-lag circuitry. The input-referred current noise of the system was 8 pA rms in a 10 kHz bandwidth and there was less than −40 dB of cross talk between the two channels. The power consumption of the device was 5 mW per channel. A leak compensation circuit, an input filter and an output filter were also integrated into the system. We have demonstrated the capabilities of the system by recording $Na_V1.7$ sodium currents from HEK 293 cells. This accurate, low-noise system can be used with planar electrodes to produce massively parallel high-throughput patch-clamp systems that can make recordings from 384 or more cells simultaneously.



3133-Pos

Effects of Continous Electrical Field Stimulation and Hypertrophic Stimulation on Micropatterned Cardiac Myocytes

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Neonatal ventricular myocytes (CM) have long been used as an *in vitro* model for hypertrophy studies. In conventional 2D culture, CM lack axial orientation and rhythmic electrical stimulation. Micropatterned cultures can restrict cell attachment to narrow stripes, leading to enhanced axial orientation, particularly in spontaneously contracting CM (Rohr et. al., Circ Res, 1991). In this study, we investigated the effect of continous electrical field stimulation (CES) on micropatterned CM and examined their response to hypertrophic stimulation. Rat CM plated in serum-containing media selectively attached to stripes (100 μm x 10 mm) of fibronectin (FN) that were microcontact-printed onto coverslips. CM cultures were subjected to CES (1 Hz, 5 V/cm) for 48 hrs, with the current applied parallel or perpendicular to FN stripes. To induce a hypertrophic response, micropatterned CM were incubated for 48 hrs in serum-free medium with the α_1 adrenoceptor agonist phenylephrine (PE, together with timolol). We determined that the size, minor/major axis ratio and angles relative to FN stripes of DAPI-stained nuclei can be used as surrogate measures of CM size, elongation and alignment, respectively. Compared to unpaced CM, parallel CES increased nuclear size (1028 ± 121 vs. 798 ± 87 μm^2, $P<0.001$), elongation (minor/major axis: 0.76 ± 0.10 vs. 0.84 ± 0.08, $P<0.001$) and alignment ($P<0.001$, Mardia-Watson-Wheeler circular statistics). Perpendicular CES caused similar but significantly less pronounced changes. PE stimulation increased nuclear size (809 ± 93 vs. 682 ± 99 μm^2, $P<0.05$), but did not increase elongation or alignment with or without CES. In conclusion, CES can be used to enhance the degree of differentiation of micropatterned CM due to continuous electrical activation and/or contractions and does not interfere with their hypertrophic response. Continuously paced micropatterned CM represent an advanced model for the investigation of hypertrophic responses and mechanisms and may be suitable for other applications.

3134-Pos

Epithelial Coating Mechanisms by Semi-Solid Materials: Application to Microbicide Gels

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Many epithelial surfaces have natural coating by polymeric materials, e.g. mucus. Foreign materials may be introduced for coating, e.g. for lubrication or drug delivery: examples are vaginal gels delivering mucosal antigens or topical microbicides. We present here a next generation biophysical vaginal coating model, which supersedes our previous work. The model characterizes the vagina as an elastic tube with a flattened lumen. The walls have porous surfaces through which natural vaginal fluid transudates, contacting and diffusing into a gel coating layer within the lumen. Spreading of the gel layer is driven by gravity and other trans-luminal pressure gradients, and wall elasticity. Gel rheology is characterized by the Carreau constitutive equation, including the presence of a yield stress. The model determines the local dilution of gel as water is transported into it, which is linked to local dilution and time-dependent rheological properties. This association is obtained experimentally. Gel coating flow is computed, accounting for variable properties at each spatial location and time step. A set of current and prototype microbicide gels is being evaluated. Results show the predominance of yield stress at later times during flow; the flow ceases when remaining vaginal wall distension is insufficient to develop shear stresses that exceed the yield stress. Dilution is most important near the vaginal walls and the leading edge of the spreading bolus. It is there that dilution proceeds most quickly, where the local viscosity of the gel drops most, and where spreading accelerates most. For the test gels, there are tradeoffs amongst the dilution-dependent yield stress, limiting low shear viscosity, and rate of shear thinning, in rates of epithelial coating. Practically, these provide flexibility in optimizing gel compositions for target rates of epithelial coating. [Supported by NIH AI48103, CHRP ID07-B-135]

3135-Pos

Mussel-Inspired Self-Healing Hydrogels

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The strength of the coordinate bonds in metal-ligand coordination complexes combined with their capacity to reform after breaking has been proposed as a source of the high toughness and potential self-healing of certain natural materials. Several studies have aimed at testing the mechanical properties of solid-state materials crosslinked with tris-catechol-Fe^{3+} complexes. However, due to the low solubility of Fe^{3+} at high pH, these studies have been performed at low pH favoring mono-catechol-Fe^{3+} complexes and at Fe:catechol ratios >> 1/3, in disagreement with the stoichiometry of tris-catechol-Fe^{3+} complexes. The tough outer cuticle of mussel holdfast threads has recently been shown to be crosslinked by tris-catechol-Fe^{3+} complexes, in agreement with the alkaline pH of seawater (pH 8). Inspired by the likely pH changes in the secretory pathway of mussels we demonstrate that a concentrated solution of a simple polymer modified with catechol and mixed with Fe^{3+} at a Fe:catechol ratio of 1/3 at pH 3 instantly gels via tris-catechol-Fe^{3+} crosslinking upon raising the pH 9. The resulting gels have strengths comparable to covalently cross-linked gels (~ 10^3-10^4 Pa) but with an order of magnitude higher energy dissipation as well as the capacity to self-heal.

3136-Pos

Hydrogel for *in Situ* Encapsulation of Multiple Black Lipid Membranes

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Hydrogels are hydrophilic, porous polymer networks that can absorb water up to thousands of times their own weight. They have many applications, one of which is the encapsulation of free-standing black lipid membranes (BLMs) for novel separation technologies or biosensor applications. We investigated gels for *in situ* encapsulation of multiple black lipid membranes across apertures in a hydrophobic ethylene tetrafluoroethylene (ETFE) support. These gels consisted of networks of poly(ethylene glycol)-dimethacrylate or poly(ethylene glycol)-diacrylate polymerized using either a chemical initiator or a photoinitiator. The hydrogels were studied with regard to their material properties such as chemical resistance, swelling behaviour, water permeability and porosity. We found that lifetimes of membranes in gel precursor solutions were short compared to lifetimes in buffer. However, crosslinking the gel stabilized the membranes and increased BLM longevity substantially over lifetimes in buffer. Optical images of the membranes and incorporation of the transmembrane peptide gramicidin A showed that the lipid membranes retained their integrity after encapsulation with hydrogel.

3137-Pos

Fabricating 3d Ordered Cell Culture Matrix by Microfluidic Device

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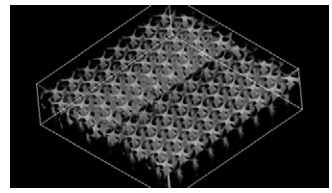
We create a novel 3D matrix with uniform and ordered pores by microfluidic technique. We can vary the pore size and the interconnection between the pores andmeasure the elastic modulus of the matrix. We culture cells inside and observe their morphology by confocal microscopy. Our matrix allows 3D cell cultures in a uniform environment.



3138-Pos

Affinity Baits and the Interior Environment of Hydrogel Particles

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Low molecular weight peptides and proteins can provide biomarkers that are diagnostic for diseases such as cancer. Unfor les can be difficult to isolate and analyze, hampering the identification of suitable biomarkers for many diseases. Thermoresponsive hydrogel particles based on a cross-linked poly(N-isopropylacrylamide), pNIPAm, architecture can be used to harvest biomarkers from biological fluids. The hydrogel particles sequester and concentrate low abundance low molecular weight analytes that can be subsequently analyzed using methods such as mass spectrometry. Introduction of monomers such as acrylic acid and allylamine into the pNIPAm skeleton allows the particles to preferentially attract and concentrate analytes based on charge. Moreover, affinity dyes, such as Cibacron Blue F3G-A, have been added to enhance the harvesting capabilities of particles.[1]

Particles based on pNIPAm shrink and swell in response to changes in environmental conditions, such as temperature, pH and salt concentration. As the pNIPAm particles shrink, their interior environment becomes more hydrophobic, which likely impacts their binding, sequestration and release properties. Here, a phenolphtahlein uptake assay has been used to monitor the interior environment of pNIPAm-based hydrogel particles, and how it changes in response to alterations in the exterior environment. The study focuses on particle responses to changes in the concentration of salts that have been shown to impact particle size, such as guanidinium chloride and ammonium sulfate.