The effect of aperture size on gigaseal formation

Conference or Workshop Item

Micro- and Nanotechnology II

3457-Pos Board B612

Dual-Pore Glass Chips for Single-Channel Recording

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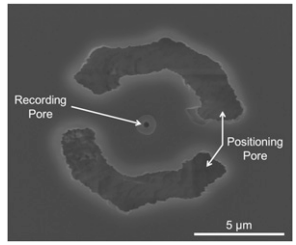
Despite the widespread use of high-throughput planar patch-clamp instruments, the conventional pipette-based technique remains the method of choice for recording single-channel activity. Generally, planar platforms are not well suited for single-channel studies due to excess noise resulting from low seal resistances and the use of substrates with poor dielectric properties. Since these platforms typically use the same pore to position a cell by suction and establish a seal, biological debris from the cell suspension can contaminate the pore surface prior to seal formation, thereby reducing the seal resistance. Here, femtosecond laser ablation is used to fabricate dual-pore glass chips for use in low-noise, single-channel recordings that circumvent this problem. One pore positions a cell by suction while another nearby pore, the recording pore, avoids contamination by maintaining positive pressure until a cell is positioned and then establishes a seal. Taking advantage of the high seal resistances and low capacitive and dielectric noise realized using glass substrates, patch-clamp experiments with these dual-pore chips consistently achieved high seal resistances (>10 GΩ), maintained gigaseals for prolonged durations (up to 6 hrs), and enabled single-channel recordings in cell-attached mode that are comparable to those obtained by conventional patch-clamp.



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The Effect of Aperture Size on Gigaseal Formation

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Patch clamping, the gold standard for ion channel studies, is entirely dependent on formation of a high resistance seal between cell membrane and patching site, known as gigaseal. As this process is laborious and time consuming, there have been many attempts to develop automated high throughput chip-based patch clamping devices. In spite of recent advances, these devices still cannot form gigaseals relying instead on less tight MΩ seals that impede their ability to measure the pA ionic currents passing through single ion channels. Progress is presently limited due to a lack of understanding of the physical and chemical mechanisms underlying gigaseal formation. In all forms of patch clamping access to the cell is achieved via a small aperture. Here, we systematically examine the influence of aperture size, micropipette rim morphology, and surface roughness on gigaseal formation in conventional patch clamping using micro/nanofabrication and modelling techniques. Our results show that smaller aperture sizes lead to improved seal formation within a range of x-y. For aperture sizes out of this range, either bigger or smaller, gigaseal formation is very difficult if not impossible. While in the literature the surface quality of patching sites is only described by average surface roughness, this research reveals that parameters such as: developed interfacial area ratio, valley void volume of the surface, ratio of core void volume to core material volume, and maximum peak to valley distance play more important roles in seal formation. Furthermore, these parameters are size dependent; as a result glass micropipettes with smaller aperture sizes are flatter and have lower water retention ability resulting in better seals. Results of this work support the practical knowledge that pipettes having smaller apertures form better seals.

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Molecular Nanotweezers; True Nano-Manipulation of Macromolecules and Other Bioparticles

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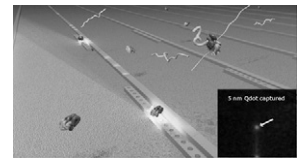
The Molecular NanoTweezer is a new system capable of optically trapping the smallest objects yet including individual proteins, quantum dots, lambda DNA, polystyrene microspheres and viruses. The key to this technology is in producing incredibly small ''spots'' of light (using near field photonics) which reach out and grab nearby particles like a tractor beam. Till now, the only direct way of altering or manipulating these objects was with chemistry. Traditional optical tweezers are unable to stably trap bioparticles smaller than 100 nm in characteristic size due to the diffraction limit of light. Optofluidics' Molecular NanoTweezer technology is able to bypass this limitation by exploiting near field optical forces in custom photonic chips. In this talk, we will discuss the current achievements of the Molecular NanoTweezer, as well as present a brief overview of its envisioned future applications. These future applications include (1) localizing individual proteins in 3D space for single molecule researchers, (2) nanoparticle size measurements and the ability to do label-free size-based assays, (3) producing custom-made protein agglomerates to be used as ultra-high binding capacity beads and (4) straight-forward single particle AFM tip functionalization.



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Membrane Disruption by Silica Nanospheres is Modulated by Surface Chemistry and Biocoating

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Silica nanospheres are promising candidates for targeted drug delivery applications because mesoporous spheres loaded with small-molecule drugs can release their cargo under specific in-vivo conditions. However, intravenously introduced nanoparticles, as well as nanomaterials that have been inadvertently inhaled or ingested, may cause adverse health effects. The potential toxicity of nanoparticles is typically investigated by establishing the viability of cultured cells in the presence of nanoparticles. A common cytotoxicity assay is the lactate dehydrogenase leakage test, which indicates cell membrane damage. Although some systematic cytotoxicity studies show evidence for structure-effect correlations (e.g. positively charged particles tend to reduce viability), cytotoxic mechanisms remain elusive, also because particles in biological solutions tend to acquire a protein corona which changes their effective size and surface charge.

To gain insight into direct nanoparticle-membrane interactions, we have measured the release of a fluorescent dye from unilamellar liposomes following exposure to silica nanospheres with different diameters and various surface functionalizations. For those particles that perturb membrane structure, as quantified by the intensity of the released dye, effects are dose dependent. Significant differences were found for silica nanospheres of 50, 200 and 500 nm nominal diameter. The extent of membrane perturbation is strongly modulated by the nanosphere surface chemistry, particularly for the smallest particles, which is surprising because our zeta potential characterization suggests that the surface density of functionalized groups (amine or carboxyl) is relatively low. Varying the lipid composition of the liposomes or coating the nanoparticles with biomolecules markedly affects membrane leakage as well.

These well-defined systems reveal an interesting interplay between model membranes and nanospheres and highlight the importance of performing nanotoxicology assays in biological solution rather than buffer or cell culture medium.

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Characterization of a Protein Nanoparticle, Bsanano and its Interaction With-Emodin

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Cell suspension and invasion is a crucial step in the metastatic cascade of cancer cells, and interruption of this step is considered to be a logical strategy for prevention and treatment of tumor metastasis. Emodin is the major active component of the rhizome of Rheum palmatum L., with known anticancer activities. Herein the effects of a plant anthraquinone: emodín, bound to a BSA nanoparticle formed by γ-irradiated BSA molecules was obtained in order to produce a new possible antimetastasic drug.

BSA nanoparticles (BSAn) and Emodin, as BSAn:Emodín bioconjugate characteristics and binding activity was examined by spectroscopy and release kinetics assays. BSAn:Emodin was characterized by column chromatography, light scattering, UV-Vis, FTIR, and electron microscopy (TEM). Interaction between BSAn and Emodin was analysed by docking-release technology BSAn and Emodin binding characteristics were determined by different biophysical methods. Binding release is slower and longer controlled release than molecular BSA:Emodin complex. Being the BSA:Emodin release a two way process, and the bioconjugate a one way process.

FTIR measurements indicate an increase in the α-helical content of the protein and a change in the environment of the tryptophan residues that bury in the interior of the biomolecule. This variation on the secondary structure could