New and Notable

Patch Clamp on a Chip

Fred J. Sigworth and
Kathryn G. Klemic
Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06520 USA

To use one of the new benchtop patch-clamp systems, you first harvest the cells from a tissue-culture dish and wash by pelleting them in a microcentrifuge. Then you open the lid of the patch clamp box and pipette a few microliters of the cell suspension into the recording well. After a few minutes the cells settle onto the electrode array at the bottom of the well, the computer selects an electrode that has made a good seal, and the recording starts.

This "biochemist's patch clamp" is still a few years in the future, but in this issue of *Biophysical Journal* Fertig et al. (2002) describe an important milestone toward its development, namely the first successful demonstration of patch-clamp recordings using a micromachined glass chip. The strong economic driving force behind this and related technical developments is not necessarily to make life easier for researchers at the bench, but rather to provide a high-throughput screening tool for drug discovery efforts (Xu et al., 2001). Suppose one had electrodes at the bottom of each well of a 384-well plate, each connected to an amplifier and able to make a whole-cell patch recording. Then massively parallel screens for ion channel activity could be carried out. But we predict that a useful spin-off of this effort will also be simple benchtop patch-clamp systems.

In conventional patch-clamp recording, a glass or fused-quartz micropipette is used as the ionic electrode: it is filled with an ionic solution which electrically connects a silver-silver chloride electrode wire to a small "patch" area of cell membrane at the small opening of the pipette tip. Critical to the method is the formation of a high resistance (gigohm) seal between the pipette and the cell membrane.

For about a decade, workers in various laboratories have sought ways to replace the pipette with "planar" patch-clamp electrodes, in which a micrometer-sized hole is made in a suitable, thin insulating partition. The idea is then to place a cell over this hole (which corresponds to the pipette tip) and to fill the underside of the partition with electrode solution. A metal contact on the underside completes the patch electrode. Early attempts to make planar electrodes involved the formation of silicon oxide or silicon nitride membranes suspended over pits etched in silicon wafers. The oxide or nitride membranes were typically 0.1–1 μm thick; into them were introduced micrometer-sized holes by photolithography and etching. These structures have proven useful for recording from liposomes (Schmidt *et al.,* 2000) and painted artificial lipid bilayers (Pantoja et al., 2001), but no reports of gigohm seals or successful recordings from cells have appeared. We also failed to obtain gigohm seals with structures made in our laboratory by anisotropic etching of single-crystal quartz. It is not certain what is the difficulty with seal formation, but we speculate that the narrow sidewalls or sharp corners of the etched apertures do not provide sufficient contact area with the cell membrane to allow a seal to be formed.

For their successful planar electrodes Fertig et al., have turned to a proven material and to an electrode geometry very similar to that of conventional pipettes. Starting with fused-quartz wafers, they formed round, gradually tapering holes by ion-track etching. In this amazing process, a single high-energy gold ion is shot through a 20-μm-thick region of a wafer, leaving behind a track of molecular damage that is highly susceptible to etching by hydrofluoric acid. Exposing the inner surface of the wafer to an HF solution therefore produces a narrowly tapered cavity. When etching is stopped at the correct moment, the cavity breaks through the outer surface to produce a micrometer-sized hole.

The result of this process is an electrode with quite good properties. The authors report a very respectable 30% success rate in obtaining "blind" recordings from cell suspensions, and the mechanical stability of the system allows whole-cell recordings to be made by mechanically rupturing the cell membrane after sealing. The capacitance and access resistance of the planar electrodes is potentially superior to those properties of conventional pipettes, but the seal resistances are somewhat lower—by about a factor of 3—than those obtained with pipettes. This last property might reflect roughness of the surface after etching.

The practical application of these planar electrodes will require some further development. First, a means for fabricating arrays of identical electrodes will need to be developed. In principle this is a matter of careful process engineering like that employed in the microelectronics industry. Second, assuming that the electrodes will not be inexpensive to make, a reliable method for cleaning electrodes for reuse will need to be worked out. Finally, techniques for preparing clean cell suspensions and methods for guiding cells to the electrode apertures may

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Address reprint requests to Fred J. Sigworth, Department of Cellular and Molecular Physiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520. Tel.: 203-785-5773; Fax: 203-785-5773; E-mail: fred.sigworth@yale.edu.

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need to be developed. In this regard Fertig et al. have obtained surprisingly good results with a simple washing procedure involving centrifugation of cells and a simple cell-positioning process that makes use of gentle suction through the electrode aperture.

Meanwhile in various laboratories other materials and technologies are being pursued. Although gigohm seals on cell membranes have not to date been reported with them, thin silicon nitride membranes are still under active investigation for patch electrodes. The structures can be fabricated with relative ease, and a particularly elegant property of this electrode geometry is the high electrostatic field that appears near the hole when a potential is applied to an "open" electrode. Schmidt et al. (2000) have exploited the field to guide liposomes to the electrode aperture, and one imagines that small cells might be guided in the same way.

A promising alternative material for planar patch electrodes is polydimethylsiloxane (PDMS), a silicone elastomer that is well known to the patch-clamp community under Dow Corning's trade name Sylgard. PDMS can readily be molded with sub-micrometer features; its surface can also be modified by plasma oxidation to make it hydrophilic. It turns out that the modified surface forms gigohm seals with cell membranes. Macropatch recordings from *Xenopus* oocytes have recently been made from micromolded PDMS electrodes and arrays (Klemic et al. 2002).

While much activity has focused on planar electrode arrays, the automation of patch-clamp recording is also being pursued with the traditional glass-pipette technology. The company Sophion in Denmark has developed a robotic patch-clamp system in which machine vision is used to locate cells under a microscope, position the pipette and establish recordings. In the instrument developed by the company CeNeS, cells are suspended in a drop of solution, and a pipette blindly approaching the drop from below encounters cells concentrated near the air-water interface.

The paper by Fertig et al. in this issue represents an important step in the race to develop a high-throughput patch-clamp system for the pharmaceutical industry (Xu et al., 2001). Such a system should be useful also in proteomics, for example in screening for new ion channel subunits in expression libraries. We expect that the benefits of the new technologies will also trickle down to the biophysical community. Planar electrodes from materials like quartz and PDMS promise to provide higher-resolution recordings, due to their potentially lower capacitance, access resistance, and dielectric noise. Transparent planar electrodes will also be well suited for optical measurements, making possible for example simultaneous single-molecule electrical and optical recordings. And in the not-too-distant future the new electrode technologies may greatly simplify the technique of routine patch-clamp recording.

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