

Micromolded PDMS planar electrode allows patch clamp electrical recordings from cells

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Abstract

The patch clamp method measures membrane currents at very high resolution when a high-resistance ‘gigaseal’ is established between the glass microelectrode and the cell membrane (Pflugers Arch. 391 (1981) 85; Neuron 8 (1992) 605). Here we describe the first use of the silicone elastomer, poly(dimethylsiloxane) (PDMS), for patch clamp electrodes. PDMS is an attractive material for patch clamp recordings. It has low dielectric loss and can be micromolded (Annu. Rev. Mat. Sci. 28 (1998) 153) into a shape that mimics the tip of the glass micropipette. Also, the surface chemistry of PDMS may be altered to mimic the hydrophilic nature of glass (J. Appl. Polym. Sci. 14 (1970) 2499; Annu. Rev. Mat. Sci. 28 (1998) 153), thereby allowing a high-resistance seal to a cell membrane. We present a planar electrode geometry consisting of a PDMS partition with a small aperture sealed between electrode and bath chambers. We demonstrate that a planar PDMS patch electrode, after oxidation of the elastomeric surface, permits patch clamp recording on *Xenopus* oocytes. Our results indicate the potential for high-throughput patch clamp recording with a planar array of PDMS electrodes. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

The patch clamp technique is the central tool for studying ion channels. It provides a voltage clamp measurement of ionic current in either a small patch of cell membrane, or the entire membrane area of a small cell (Hamill et al., 1981; Neher, 1992). Because it is a measurement of current, it directly monitors the number of active channels in the membrane, and is therefore the assay of choice for agents that block or modulate channel activity. There is tremendous interest in improving the throughput and ‘ease of use’ of the patch clamp method, primarily to facilitate drug screening in the pharmaceutical industry but also as a means to directly assay the function of the genes that encode ion channel and transporter proteins. Recent efforts to develop micromachined electrode arrays have been

successful only in recording from artificial membranes (Schmidt et al., 2000; Fertig et al., 2001).

In conventional patch clamp recording, the $\sim 1 \mu\text{m}$ tip of a glass or fused-quartz micropipette, filled with saline solution, is sealed over a patch of cell membrane, electrically isolating it with a very large electrical ‘seal resistance’ of 10–100 G Ω . Membrane currents are recorded through a sensitive current-to-voltage converter connected to an Ag–AgCl electrode in the micropipette. Currently, this method is not practical for high-throughput screening because it requires a skilled operator to manually manipulate the glass pipette onto the cell. In addition, the irreversible nature of the sealing process requires a new pipette be used for each recording.

Here we describe the first use of a new material and a new configuration for patch clamp electrodes. A planar electrode is micromolded from the silicone elastomer, poly(dimethylsiloxane) (PDMS). PDMS, also known as Sylgard® (Dow Corning Corp.) is a popular material for micromolding and microfluidics applications (Xia and Whitesides, 1998). It has excellent dielectric properties

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that would recommend it as a patch electrode material, except that its hydrophobic surface prevents the establishment of a high-resistance seal with cell membranes. The surface chemistry of PDMS, however, may be altered to mimic the hydrophilic nature of glass (Xia and Whitesides, 1998; Hollahan and Carlson, 1970). We found that a PDMS coating on a glass micropipette tip, once oxidized, readily forms gigaohm seals. To take full advantage of the material, we proceeded to make electrodes in which a planar PDMS partition replaces the micropipette.

2. Materials and methods

2.1. Fabrication of PDMS partition

PDMS partitions were fabricated by two methods, micromolding from a quartz rod pulled into the shape of a micropipette or micromolding from a micromachined silicon master.

The first method of fabrication followed the two-step process shown in Fig. 1a. In the first step a solid quartz rod pulled into the shape of a conventional micropipette (Penner, 1995) was used as a master mold. PDMS was poured around the rod and cured at 65 °C for 4 h. The quartz rod was removed, leaving a hollow cavity in the PDMS block. Next sections, 200 µm thick, were cut from the block using a laboratory microtome. Each section had a small aperture ranging between 2 and 20 µm in diameter (see Fig. 1b and c). Each PDMS section was glued onto a plastic coverslip for support, centering its small aperture in a 1-mm hole punched in the coverslip.

We used the poly(dimethylsiloxane) produced by Dow Corning, Sylgard® 184 (Essex/Brownell Products, Edison, NJ). Glass pipettes used for micromolding were fashioned using the P2000 Laser Micropipette puller (Sutter Instruments, Novato, CA), using standard methods (Penner, 1995). Essentially, the micropipette puller heats the center of a quartz rod (1 mm dia., Sutter Instruments, Novato, CA) with a laser while pulling the ends until the quartz breaks, leaving two identical sharp tip quartz rods. The PDMS mold cast from the quartz micropipette was sliced using a Leica VT1000S microtome (Leica Microsystems, Heidelberg, Germany).

The second method of fabrication used a micromachined silicon wafer for the first stage of micromolding. A 4 × 4 array of 400 µm deep, self-terminated pits spaced 1 mm apart were anisotropically etched in a silicon wafer. That shape was then transferred through two PDMS molding steps into an array of pyramids in polyurethane, see Fig. 2a. An important step in forming the PDMS in step 4 of the figure was the plasma treatment of the first PDMS mold so that the two PDMS layers could be separated. The final PDMS

electrode partition was formed by pouring freshly prepared PDMS onto the polyurethane mold and then capping the polyurethane pyramids with a glass slide (step 6). The ultraviolet-curable polyurethane used for molding was Epo-Tek OG169 (Epoxy Technology, Billerica, MA). The contact between the tip of the polyurethane pyramid and the glass slide excluded the PDMS to form small apertures (Fig. 2c and d). The size and shape of the aperture depended on the amount of contact with the glass slide. We chose polyurethane because it deforms sufficiently when contacting the glass slide to make holes 4–20 µm in size.

2.2. Surface treatment

For most surface treatments, we used the Balzer Med 010 sputtering system (Bal-tec, Manchester, NH) to plasma-treat the surface of the PDMS by a 10 min exposure to a high voltage (600 V) discharge in 1 mTorr of air. For measurements using the PDMS electrode array, the PDMS surface was exposed to a 15 min oxygen plasma generated at 100 W and 700 mTorr, using the Anatech SP100 plasma system (Anatech LTD, Springfield, VA). All samples were used within a half-hour of plasma treatment if stored in air and within 2 h if stored in water.

2.3. Oocyte preparation and RNA injection

The oocytes were harvested and maintained from *Xenopus laevis* frogs as previously described (Stühmer, 1998). The channel cDNA construct (Mathur et al., 1997) contained an N₆ terminal deletion to remove inactivation (Hoshi et al., 1990) and was contained in the pGEM vector. In vitro transcribed RNA was prepared using standard techniques (Stühmer, 1998) and injected into each oocyte using a microinjector (Drummond Scientific Co., Broomhall, PA).

2.4. Electrical measurements

Patch clamp measurements were made in the ‘cell-attached’ configuration. A devitellinized oocyte Methfessel et al. (1986) was dropped onto the aperture from a Pasteur pipette containing bath solution. Data shown in Fig. 3 was recorded using an Axon 200B patch clamp amplifier (Axon Instruments, Union City, CA), digitized at 20 kHz following a 2 kHz filter using the Instrutech ITC-18 (Mineola, NY) analog interface and using Pulse software (HEKA-Electronic, Lambrecht, Germany). Data shown in Fig. 4 was recorded with the Multiclamp 700A (Axon Instruments), digitized at 20 kHz following a 1 kHz filter using the Digidata 1322A (Axon Instruments) analog interface and using pClamp v8 software (Axon Instruments). A multiplexer chip (MAX336, Maxim Integrated Products, Sunnyvale, CA) allowed

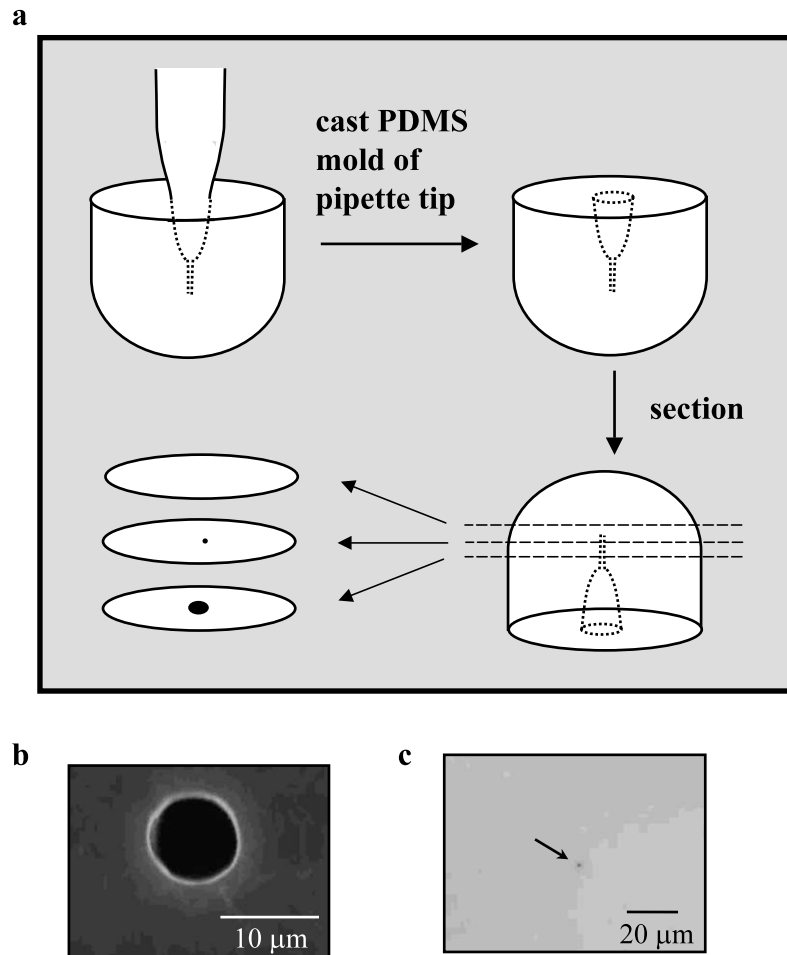


Fig. 1. Fabrication of planar PDMS patch electrode. (a) Schematic of the two-step process for making a planar PDMS patch electrode. First, a PDMS mold is cast from a solid quartz rod, in the shape of a standard micropipette. Then the PDMS mold is sectioned into disks of 200 μm thickness. (b) SEM photo of a PDMS partition having a large (10 μm) molded aperture. (c) Light micrograph of another partition with a small aperture ($\sim 2 \mu\text{m}$).

individual electrodes in the array to be connected to the amplifier input.

The electrode and seal resistance was calculated from the mean current measured in response to an applied 5 mV, 10 ms pulse. Seal formation was monitored by repeated measurement of the resistance as calculated by the software. Suction was applied to conventional patch pipettes via a tube connected to a 10 ml syringe.

Bath and electrode solutions were respectively (in mM): 130 K-Aspartate, 10 KCl, 1 EGTA, 10 HEPES; and 140 K-Aspartate, 1.8 CaCl_2 , 10 HEPES. All chemicals were purchased from Sigma Chemical Co. (St. Louis, Missouri).

2.5. PDMS coating on conventional patch pipettes

Conventional patch pipettes were fabricated from borosilicate capillary glass (Warner Instruments, Hamden, CT) in the standard fashion (Penner, 1995). Some patch pipettes were coated to the tip with freshly prepared PDMS. By capillary action, PDMS entered

the tip of the pipette to coat the inside of the tip. The aperture was then cleared of PDMS by forcing air through the pipette with a syringe connected via tubing to the back end of the pipette. The PDMS was then cured by placing a hot tungsten filament near the tip of the pipette, while maintaining the positive airflow from the syringe. This resulted in a thin PDMS coating of both the inside and outside of the pipette tip. Another layer of PDMS was then added to the shank of the pipette and heat cured to reduce electrode capacitance.

3. Results

3.1. PDMS-coated patch pipettes

A successful patch clamp measurement requires that a tight electrical seal forms between the electrode and the cell membrane, termed a ‘gigaseal’ as the resistance must be at least one gigaohm. While the exact mechanism of sealing is not well understood, this mechanically and

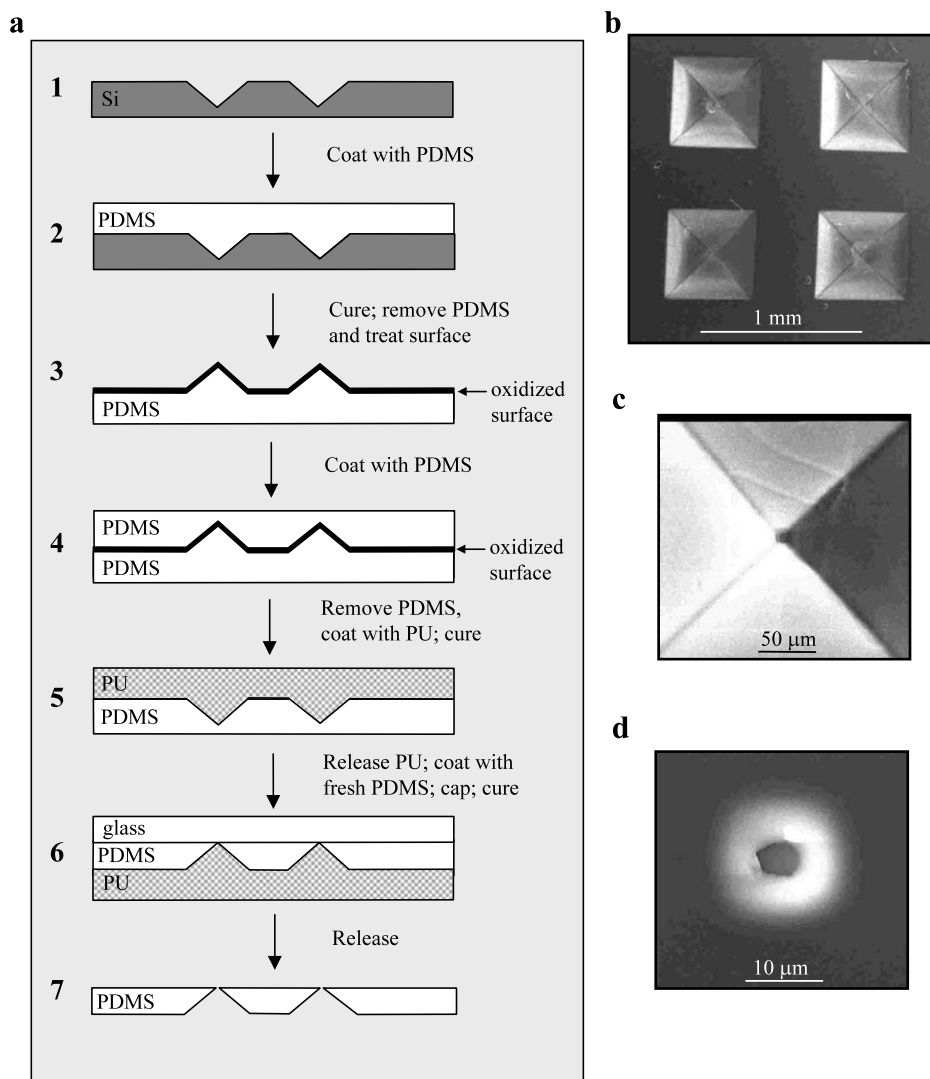


Fig. 2. Fabrication of planar PDMS patch electrode array. (a) Process steps for micromolding the PDMS partition. The process starts with a micromachined silicon master and progresses through a polyurethane (PU) intermediate mold to form the final PDMS array. (b–d) Scanning electron microscope (SEM) images of the PDMS electrode array (b), close-up of hole viewed from bottom (c) and top (d). The bright ring around the hole is a charging artifact caused by secondary electrons accumulating in the PDMS.

electrically tight seal between electrode and membrane permits high-resolution measurement of membrane currents. Therefore any material considered for use in a patch clamp measurement must form gigaseals.

To determine the feasibility of PDMS as an interface material for patch clamp measurements, we coated the tips of conventional patch pipettes with PDMS and performed seal resistance measurements on *Xenopus* oocytes, large cells 0.7–1 mm in diameter. Seal formation was monitored by repeated measurement of the electrode resistance. The electrode resistance was typically 4–10 M Ω before contacting the cell. Gentle suction was applied to facilitate gigaseal formation on all pipettes. Gigaseals were formed on five out of 13 uncoated pipettes, zero out of four PDMS-coated pipettes without surface treatment, and seven out of 13 PDMS-coated pipettes with surface treatment.

3.2. Longevity of surface treatment

The surface treatment changes the PDMS surface from a hydrophobic to hydrophilic state (Hollahan and Carlson, 1970). However, this effect is only temporary as the molecules in the polymer reorient, restoring the hydrophobic nature over time (Hillborg and Gedde, 1998; Kim et al., 1999). The changes can be monitored as a change in the contact angle of a drop of water placed on the surface. We characterized the longevity of the surface treatment for our system by monitoring the curvature of a 0.1 ml drop of water placed on the surface of a sheet of PDMS at time intervals after the surface treatment. We qualitatively ranked the ‘balling-up’ of the droplet on a scale of 1–4 and found that the time course of reversion to a hydrophobic state depends on the time of surface treatment and the environment of the

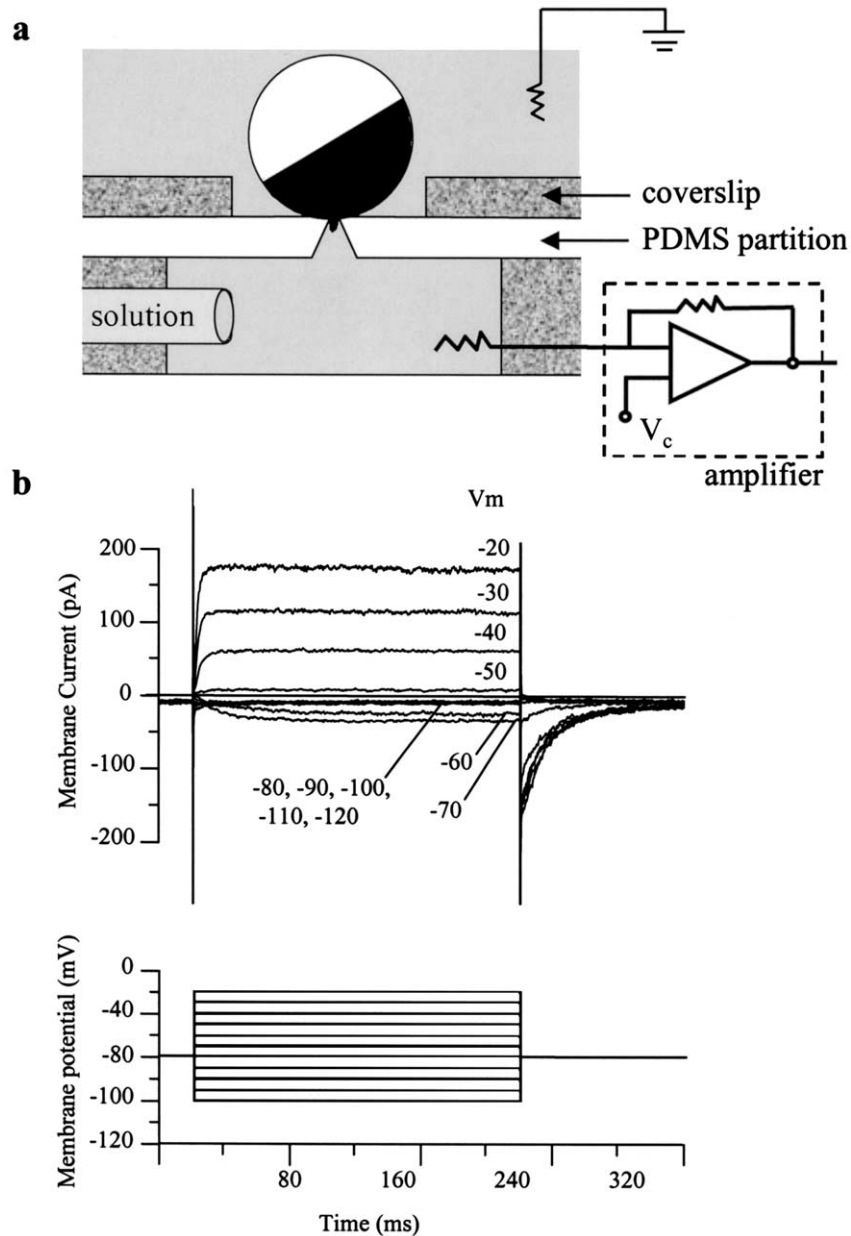


Fig. 3. Planar PDMS patch electrode recording of potassium channel currents. (a) Schematic of planar patch recording system. The 200 μm thick, oxidized PDMS partition is sandwiched between bath (upper) and electrode (lower) chambers. A devitellinized oocyte is dropped onto the aperture. The electrode chamber contains a Ag–AgCl wire connected to the input of a patch clamp amplifier; polyethylene tubing provides solution exchange. Another Ag–AgCl wire in the bath chamber serves as the ground electrode. (b) Current traces recorded from a *Xenopus* oocyte expressing inactivation-removed *Shaker* potassium channels. Each trace represents an incremental step change in membrane potential from rest, -80 mV. Voltage steps, from -120 to -20 mV in 10 mV increments, have a duration of 200 ms before returning to -80 mV. No leakage subtraction was employed. The aperture was 8 μm in diameter; initial electrode resistance was 3.2 M Ω .

sample during storage. For a 5, 10, and 15 min oxygen plasma treatment, the samples reverted with a time constant of 0.5, 0.7, and 0.9 h if stored in air and 1, 1.5, and 5 h if stored under water, respectively. For a 30 and 60 min exposure, the time constants were 1.5 and 1.8 h in air and 12 and 192 h under water, respectively. Presumably, storing the partition under water sustains the hydrophilic state by keeping the oxygen atoms from being drawn into the bulk (Kim et al., 1999).

3.3. Planar PDMS patch electrode

To use this new material for patch clamp measurements and to verify that the plasma treatment did not simply vaporize the PDMS coating on the patch pipettes, we proceeded to fabricate planar PDMS electrodes (Fig. 1a). These electrodes have an aperture size similar to the tip diameter of the glass micropipette but are formed solely from PDMS.

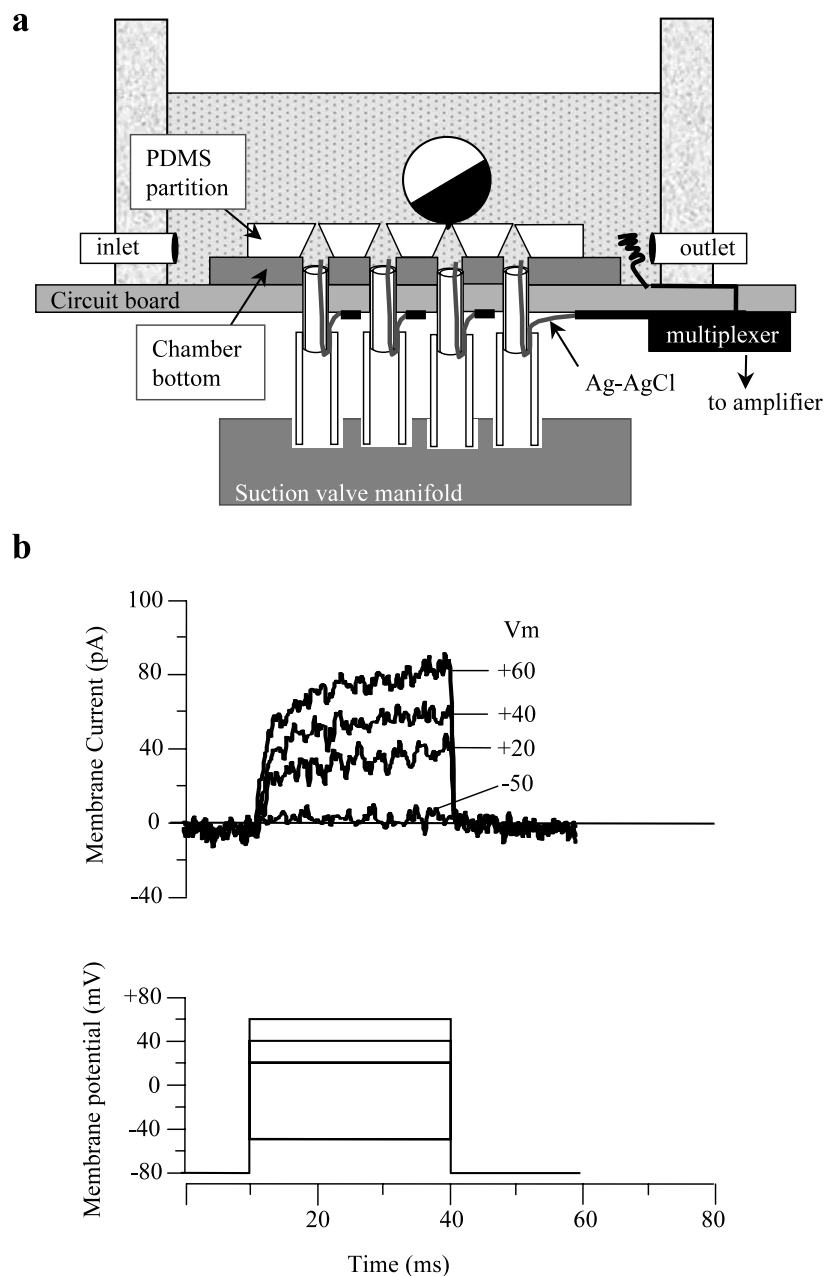


Fig. 4. Planar PDMS patch electrode array recording of *Shaker* potassium channel currents. (a) Schematic of planar patch electrode array recording system. The 400 μm thick, oxidized PDMS partition is sealed onto the chamber bottom with vacuum grease. The chamber bottom contains an array of openings, each containing tubing and a Ag–AgCl wire. The tubing connects to a suction manifold and the Ag–AgCl wire connects to a multiplexer chip on the circuit board under the chamber bottom. The multiplexer connects each contact to the amplifier electronics. Another Ag–AgCl wire in the bath solution connects to the ground of the amplifier. Bath solution is exchanged through solution lines into and out of the bath chamber. A devitellinized oocyte is dropped onto the aperture to make a patch clamp recording. (b) Current traces recorded from a *Xenopus* oocyte expressing inactivation-removed *Shaker* potassium channels. Each trace represents a step change in membrane potential from rest, -80 mV. Voltage steps are to -50 , $+20$, $+40$ and $+60$ mV, as noted, before returning to -80 mV. Leakage currents were subtracted from the raw data by a P/4 protocol (Penner, 1995).

For patch clamp experiments, the surface of the PDMS partition was plasma-oxidized and then mounted into the recording chamber (Fig. 3a). A devitellinized oocyte, expressing non-inactivating *Shaker* potassium channels (Hoshi et al., 1990), was dropped onto the aperture. Fig. 3b shows a ‘macro-patch’ recording

obtained with an 8- μm aperture. Even with this large aperture, a seal with resistance 10.2 G Ω developed spontaneously within 5 min of contact with the cell. Ionic currents carried by a population of ~ 200 *Shaker* channels were measured in response to positive step changes in membrane potential.

3.4. Planar PDMS patch electrode array

To scale up fabrication of the PDMS planar electrodes we next micromolded partitions as a 4×4 array of electrodes using a micromachined silicon structure as the master. The strategy was to use the precision of silicon micromachining to create a master structure that would define a shape similar to the tip of a glass micropipette in a thin sheet of molded PDMS. In addition, silicon micromachining provided the precision necessary to make a well ordered, 4×4 array of structures, spaced 1 mm apart. The final master structure should have a pyramidal shape about 0.4 mm high repeated across the array so that the tips of the pyramids may contact an opposing flat surface uniformly to define an array of apertures in the molded PDMS partition (Fig. 2b). Rather than machine this structure in silicon, we machined the reverse: an array of self-terminated pits. The final structure was then created in polyurethane through a series of PDMS molding steps (Fig. 2a). The polyurethane molded structure increased the life of the silicon wafer as many polyurethane copies could be made, used and discarded. In addition the polyurethane mold deformed sufficiently to make reasonably sized holes, usually between 4–20 μm (Fig. 2c and d).

For patch clamp measurements the PDMS planar electrode array was plasma-oxidized then mounted in the recording system shown in Fig. 4a. To electrically isolate each electrode, the partition was sealed onto the chamber bottom over an array of openings that each contained a Ag–AgCl wire and suction tubing. The Ag–AgCl wire connected to the multiplexer chip mounted on the circuit board under the chamber. The multiplexer then connected to the input of the amplifier. The tubing connected to a suction manifold that permitted individual or global suction.

The electrode resistance values were measured to confirm that the electrodes were electrically isolated and that the apertures were open. Fig. 4b shows a ‘macro-patch’ recording obtained when a devitellinized oocyte, expressing *Shaker* potassium channels, was dropped onto an aperture. The ionic currents carried by a population of ~ 80 *Shaker* channels were measured in response to positive step changes in membrane potential.

As is standard practice in the use of glass pipette electrodes, we used each planar PDMS electrode or array only once. The overall success rate for forming a gigaseal on a *Xenopus* oocyte using a planar PDMS patch electrode was 13% (10 out of 75). This is lower than the $\sim 40\%$ success rate for the conventional patch electrodes with or without PDMS coating and surface treatment. We expect that the success rate with planar electrodes may be improved through optimizing the aperture size and geometry.

4. Discussion

The high fidelity of patch clamp measurements requires a tight electrical connection between the cell membrane and the surface of the recording electrode. In the past, this has only been possible with glass or fused-quartz electrode surfaces. Recently, several groups have reported work using planar electrodes for measuring ion channel currents using holes etched in a thin nitride film suspended over a silicon pit (Schmidt et al., 2000) or holes etched by ion track milling in quartz (Fertig et al., 2001). To date, these electrodes have not made gigaseals to cell membranes; thus their use is limited to artificial lipid bilayer measurements. We now report that the silicone elastomer, PDMS, once plasma-oxidized, forms a ‘gigaseal’ to a cell membrane and therefore permits patch clamp recordings.

In general, a planar electrode has many advantages over the conventional patch pipette electrode. First, the planar electrode can be easily scaled to an array of electrodes to permit multi-cell or multi-site measurements. Secondly, the planar electrode has the potential for integrating microfluidic lines that would permit exchange of the electrode solution, something rarely achieved with a micropipette. The amplifier electronics could also be integrated into the substrate of the electrode for improved noise performance. Lastly, a planar electrode could be mounted over a fluorescence microscope to simultaneously record electrical and optical signals.

Micromolding a planar electrode from PDMS has advantages over the other microfabricated planar electrodes under development (Schmidt et al., 2000; Fertig et al., 2001). First, the fabrication is simple and economical so that the electrodes are disposable, therefore eliminating the difficult step of cleaning the device. Second, microfluidic lines can be micromolded in the same structure or added by bonding PDMS layers (Xia and Whitesides, 1998). Thirdly, PDMS is optically transparent so that alignment to underlying components can be done visually. The transparency of the material may also be advantageous when integrating an optical recording system. Lastly, the PDMS surface can be chemically modified to facilitate seal formation.

The probe nature of the conventional patch pipette still has its advantages. Most importantly, it can penetrate the top layers of tissue to permit patch clamp measurement on a cell within a tissue. Second, the electrode can be pulled away from a cell to make an ‘excised patch’ recording, useful for applying modifying agents to the patch of membrane. Lastly, the pipette can be moved quickly between lines of solution flow for quick changes in modifying agents. Integrated microfluidics may negate the need for these last two points but more complicated micromachining may be required to

fabricate planar patch electrodes that can penetrate tissue.

The need to plasma-treat the PDMS surface just before use may be a disadvantage of the planar electrode. This would add a plasma system to the required equipment for patch clamp experiments. However, the planar electrodes do not require use of the high-quality optical microscope and micromanipulators currently required for patch clamp recording. In addition, conventional patch pipettes commonly require several processing steps before use including heat-polishing and coating.

Interestingly the need for surface treatment of PDMS follows the current theory for how a gigaseal forms. It is believed that the cell membrane adheres to the charged surface of the glass pipette tip. This is also consistent with our observation that PDMS-coated pipettes without surface treatment actually resisted gigaseal formation. With such pipettes, we found that applying suction increased the seal resistance only to a limited extent, to values below 100 M Ω .

The major disadvantage of the planar patch electrodes described in this paper is that the aperture size was large, typically 4–20 μm . This has two consequences. First, the large size limits the use of these PDMS electrodes to large cells. Smaller cells, such as many mammalian tissue culture cells, have a diameter on the same order as the aperture. Therefore these cells do not make gigaseals to the electrode and often pass through the aperture when suction is applied to initiate gigaseal formation. Second, large aperture sizes generally reduce the probability of gigaseal formation (Penner, 1995). However, a smaller aperture also increases the series resistance and limits the ability to voltage clamp the membrane. The general rule is to have a 1–2 μm aperture that can easily form a gigaseal and still have a reasonably low resistance value. We expect that smaller apertures molded in PDMS should improve our success in forming a gigaseal and permit measurements on smaller cells of interest.

PDMS can be readily micromolded (Xia and Whitesides, 1998), yielding feature sizes well below 1 μm . We have recently redesigned our microfabricated structures to make well-defined 2 μm apertures uniformly across a 4 \times 4 array. With a uniform array of small apertures, a high density of mammalian cells could be dropped onto the array to greatly improve the success of gigaseal formation and increase the throughput of patch clamp measurements. The resulting ability to make simulta-

neous patch clamp recordings from many cells would greatly aid the discovery of new ion channel genes and new pharmacological agents directed to ion channel targets.

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