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Pressure polishing: a method for re-shaping patch pipettes during fire polishing

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Abstract

The resolution of patch-clamp recordings is limited by the geometrical and electrical properties of patch pipettes. The ideal whole-cell patch pipette has a blunt, cone-shaped tip and a low resistance. The best glasses for making patch pipettes are low noise, low capacitance glasses such as borosilicate and aluminasilicate glasses. Regrettably, nearly all borosilicate glasses form pipettes with sharp, cone-shaped tips and relatively high resistance. It is possible, however, to reshape the tip during fire polishing by pressurizing the pipette lumen during fire polishing, a technique we call 'pressure polishing.' We find that this technique works with pipettes made from virtually any type of glass, including thick-walled aluminasilicate glass. We routinely use this technique to make pipettes suitable for whole-cell patch-clamp recording of tiny neurons (1–3 μm in diameter). Our pipettes are made from thick-walled, borosilicate glass and have submicron tip openings and resistances <10 MΩ. Similar pipettes could be used to record from subcellular neuronal structures such as axons, dendrites and dendritic spines. Pressure polishing should also be useful in patch-clamp applications that benefit from using pipettes with blunt tips, such as perforated-patch whole-cell recordings, low-noise single channel recordings and experiments that require internal perfusion of the pipette. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Patch clamp; Pipette fabrication; Fire polishing; Microforge; Pipette glass

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1. Introduction

In many types of patch clamp experiments, it is the patch pipette rather than the electronics that determines the quality of the recording. An ideal whole-cell patch pipette has a low series resistance and a small capacitance that is easily compensated. Two main types of glass are available for making patch pipettes: low softening point or 'soft' glasses (such as soda lime) and high softening point or 'hard' glasses (such as borosilicate). Pipettes made from soft glass can be fire-polished so that the pipette walls converge to form a blunt, cone-shaped tip with a high cone angle. This shape reduces pipette resistance. Unfortunately, soft glass pipettes generate larger capacity transients than hard glass pipettes because soft glass has a higher dielectric constant than hard glass. In addition, the high dielectric loss factor of soft glass distorts the time-course of capacity transients, leading to difficulties in capacity compensation using conventional patch-clamp amplifiers (Rae and Levis, 1992). Although pipettes made from hard glass have smaller capacity transients that are easier to compensate, hard glass pipettes tend to form sharp tips with low cone angles and higher series resistance. Thus, there is a clear trade-off between tip geometry and electrical properties.

Here, we describe a simple way to circumvent this trade-off. We find that high cone angles can be obtained in pipettes made from virtually any glass by pressurizing the pipette internally during fire polishing, a technique we call 'pressure polishing'. The technique is applicable to both borosilicate and aluminasilicate pipettes and is unaffected by the presence of a filament. Pressure polishing works with thin- or thick-walled glass, the latter eliminating the need for coated pipettes in some cases. This technique is a practical method for

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making pipettes with submicron tip openings that have resistances < 10 MΩ. Such pipettes are essential for recording from tiny cells < 3 μm in diameter (Goodman et al., 1998) and may also find application in recording from subcellular neuronal structures including dendrites, axons and even dendritic spines. In addition, pressure polishing may be useful in other applications that require high cone angles, such as perforated-patch whole-cell recordings, low-noise single channel recordings and experiments that require internal perfusion of the patch pipette.

2. Methods

Pressure polishing is done on a conventional patch-pipette microforge (e.g. ALA Scientific CPM–2) that uses a heated platinum filament to melt glass. The microforge should be fitted with a high power objective (Nikon ELWD 100X/0.8, 2-mm working distance) so that one can observe subtle changes in pipette shape during polishing. The pipette is mounted in an electrode holder with a side or rear port that is connected to a regulated source of pressurized gas. A valve in the gas line allows the pipette lumen to be pressurized or vented to the atmosphere.

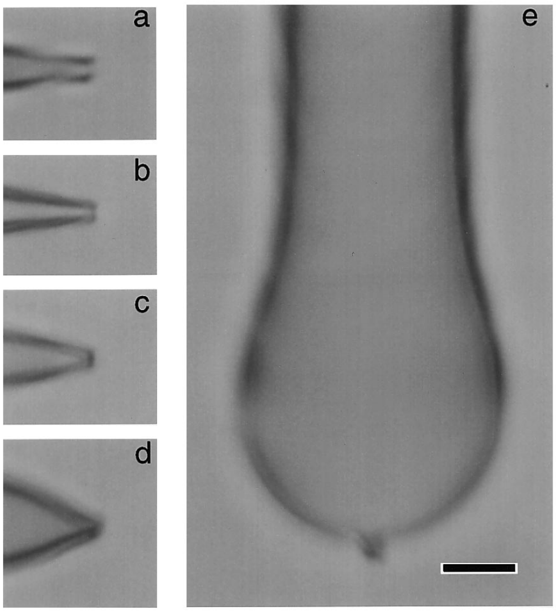

Fig. 1. A variety of tip shapes obtained by pressure polishing. (a) Unpolished pipette; (b) standard fire polishing; (c) mild pressure polishing; (d) moderate pressure polishing; and (e) extreme pressure polishing. All pipettes were pulled from borosilicate glass (BF120-69-10, Sutter Instruments) on a horizontal pipette puller (P-2000, Sutter Instruments) and polished to a final tip diameter < 1 μm. The scale bar is 10 μm.

2.1. Pressure polishing

2.1.1. Step 1

To shape the final ≈ 30 μm of the pipette tip, clean, pressurized gas (40–60 PSI ≈ 275–415 kPa) is directed into the pipette lumen and the pipette is heated until the walls expand to the desired shape, at which time the heat is turned off and the pipette is allowed to cool.

2.1.2. Step 2

The pipette is fire polished without pressure to reduce the diameter of the opening. The second step can be omitted to produce pipettes that have a high cone angle but a larger tip diameter. This approach may be particularly useful for perforated-patch clamp recordings.

3. Results

Fig. 1 shows a comparison of pipette shapes obtained by conventional and pressure polishing techniques. Pipettes were pulled from thick-walled borosilicate capillaries (BF120-69-10, Sutter Instruments, Novarto, CA) in a standard electrode puller to an initial tip opening of 2–6 μm. One can see that conventional fire polishing reduces the diameter of the tip opening without significantly changing the cone angle over the first 10–15 μm (Fig. 1a,b). In pressure polishing, however, the pipette walls expand as the glass is heated, resulting in a tip with a higher cone angle (Fig. 1c). A wide range of cone angles (Fig. 1c–e) can be obtained by varying the duration of the pressure polishing step or by increasing the pressure applied to the pipette lumen. Similar results can be obtained with soda lime, thin-wall borosilicate, and thick-wall aluminasilicate pipettes with and without filaments.

Pipette resistance under four different polishing conditions is shown in Table 1. Pipettes were again pulled from thick-walled borosilicate capillaries (BF150-86-10, Sutter Instruments). Our results show that for a given tip diameter, pressure polished pipettes have substantially lower resistances than pipettes that were not pressure polished, presumably because of the differences in cone angles at the tip. This result is consistent with the observation, reported previously (Sakmann and Neher, 1995), that a significant proportion of overall pipette resistance is concentrated at the tip.

4. Discussion

Choosing glass for patch-clamp recording generally involves striking a balance between the desired tip geometry and electrical properties. A common resolution of this dilemma has been to choose hard glass in experiments where electrical performance is more im-

Table 1
Pressure polishing vs. conventional polishing

Pipette treatment[a]	Tip opening (μm)	R (MΩ)	Relative R[b]
Unpolished	1.4 ± 0.2 (5)	3.1 ± 0.3 (13)	1
Fire polished (conventional)	<0.7	13 ± 2 (7)	4.2
Pressure polish: Step 1 only	1.3 ± 0.3 (5)	2.4 ± 0.3 (12)	0.77
Pressure polish: Steps 1 and 2	<0.7	5 ± 1 (6)	1.6

[a] Pipettes were pulled from BF150-86-10 and pulled on a P-97 Pipette Puller (both from Sutter Instruments) and filled with a physiological, intracellular saline composed of (in mM): K gluconate (125), KCl (18), NaCl (2), $CaCl_2$ (0.7), $MgCl_2$ (2) K_2EGTA (10), KHEPES (10) pH 7.2. To measure resistance, pipettes were immersed in an extracellular saline composed of (mM): NaCl (145), KCl (5), $CaCl_2$ (1), $MgCl_2$ (5), NaHEPES (10), D-glucose (20), pH 7.2.

[b] To facilitate comparison, resistance was normalized to the average resistance of unpolished pipettes.

portant than tip geometry and to choose soft glass in experiments where tip geometry is more important than electrical performance. Until now, the primary means for maximizing the electrical performance of pipettes with blunt tips (made from soft glass) has been to coat the tip with a substance that has better dielectric properties than soft glass (e.g. wax or Dow-Corning Sylgard™). As reported previously (Rae and Levis, 1992), this approach is only partially effective. A less-used solution is to use low softening temperature, high-lead borosilicate glass. Although high-lead glasses (e.g. Corning 8161) produce pipettes with high-cone angle tips and good electrical properties, these glasses contain leachable components that can block ionic currents (Cota and Armstrong, 1988; Furman and Tanaka, 1988). Pressure polishing thus emerges as an important new way to circumvent the trade-off between the geometrical and electrical properties of patch-clamp pipettes. By coating pressure-polished, hard glass pipettes, it may be possible to obtain pipettes with superior electrical characteristics as well as an optimal shape.

The main effect of pressure polishing is to increase the cone angle at the tip of the pipette. An important result of high cone angle is reduced series resistance, the benefits of which are threefold. First, it results in smaller errors in the voltage clamp signal due to the voltage drop across the pipette resistance. Second, it reduces the filtering of current clamp signals caused by the interaction between series resistance and cell capacitance. Third, it reduces the component of electrical noise that is proportional to the product of series resistance and cell capacitance (RC noise). This is particularly beneficial in whole-cell recordings where RC noise is the dominant noise source (Levis and Rae, 1998).

High cone angles confer several other advantages. First, a high cone angle makes it easier to obtain the whole-cell recording configuration, perhaps by allowing greater expansion of the membrane bleb within the pipette. Second, a high cone angle facilitates perforated patch recording. It both reduces the time needed for the pore-forming agent to diffuse to the pipette tip and increases the membrane area available for perforation. And, finally, pipettes with high cone angles are less likely to damage cells and fine, sub-cellular structures.

Pressure polishing also extends the range of pipette tip shapes that can be achieved in hard and soft glasses alike. For example, one can make pipettes with a nearly spherical swelling at the tip. Pipettes with a wide mouth and narrow throat can be made keeping the pipette pressurized until the sphere pops like a balloon, leaving a trumpet shaped tip. Such pipettes might be useful for grasping cells or small pieces of tissue to stabilize them or to transfer them.

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