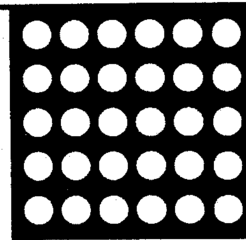
Patch-Clamp Recording



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I. Introduction

Patch-clamp recording has become the method of choice for making high-resolution functional measurements from ionic channels in the membranes of cells. Only small modifications of the basic approach allow either recordings of the currents flowing through single ionic channels or those flowing through all of the channels in a single cell. Many review articles and book chapters about patch-clamp methodology have been published previously (Hamill *et al.*, 1981; Levis and Rae, 1992; Rae and Levis, 1984, 1992a,b; Sherman-Gold, 1993; Rudy and Iverson, 1992; Standen *et al.*, 1987; Sakmann and Neher, 1983).

II. Materials and Instrumentation

Patch-clamping requires the use of an electrode puller, a dissecting microscope for use in electrode elastomer coating, an electrode tip firepolisher, a microscope with a high-quality micromanipulator, a patch-clamp amplifier with an electrode holder designed for use with the amplifier, an anti-aliasing filter, and a computer. Each of these items is obtainable from a commercial supplier. Patch-clamp amplifiers come with resistive and/or capacitive feedback elements, the capacitive feedback amplifiers having much lower noise for single-channel recording.

III. Procedures

A. PULLING ELECTRODES

1. Electrodes can be constructed from a wide variety of glass tubing. Corning 7052, available from Garner Glass, is good for single-channel recordings, whereas Kimble KG-12 from Friedrich and Dimmock is particularly useful for whole-cell recordings. Quartz tubing is best for low-noise single-channel recording but requires a P2000 laser puller from Sutter Instruments.

2. Tubing with an outside diameter of 1.65 mm and an inside diameter of 1.15 mm or so is particularly convenient. In general, the thicker the wall, the lower the noise in single-channel recordings, whereas thin walls are best for whole-cell recording.

3. The glass to be pulled should be clamped in the puller so the resulting two electrodes are the same length. Tubing to be pulled should not exceed 2.7–3.0 in. in length.

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4. Long heating filaments or coils are good for making long, slowly tapering tips, whereas short filaments or coils most easily make blunt tips.

5. Whole-cell electrodes should be blunt and low resistance (2–3 μm in tip diameter), whereas single-channel electrodes can be sharper with tips as small as 0.2–0.5 μm.

6. Pulled electrodes should not be touched near their tips with bare fingers and should be placed in closed containers to keep dust from the tips which are often statically charged.

B. ELECTRODE COATING

1. Using either a bright-field or a dark-field dissecting microscope, coat the electrode with a hydrophobic elastomer such as Sylgard No. 184 (Dow Corning) or General Electric RTV615 obtainable from Newark Electronics.

2. The painting can be done with the tapered end of a piece of glass tubing, 1.5–2.0 mm in diameter, which has been pulled in two in a Bunsen burner. Any kind of glass will do.

3. Use elastomers that have been made up according to the manufacturer's instructions, aliquoted into 1.5-ml microcentrifuge tubes and stored at −20°C. Elastomers made this way are useful for several weeks.

4. Remove a single 1.5-ml tube from the freezer but do not open it until it has reached room temperature. This keeps H_2O from condensing into the elastomer and altering its electrical properties.

5. Using the tapered glass from step 2, carefully paint the elastomer on the electrode whose tip is angled upward while observing it under the dissecting microscope. Be sure not to cover the tip with elastomer. With practice it should easily be possible to get it to within 100 μm of the tip without actually covering the tip. For single-channel recordings, background noise will fall progressively as you paint closer to the tip. In whole-cell recording, the coating allows easier cancellation of the fast capacity transient but has little effect on total background noise.

6. The elastomer can be cured by placing it, electrode tip upward, into the hot air stream of a heat gun such as a Master Model 10008, available from Newark Electronics. Five to ten seconds is required to fully cure many elastomers.

C. FIREPOLISHING ELECTRODES

1. This should be done after elastomer coating either with a commercially available microforge or with a custom constructed apparatus.

2. Either an inverted or an upright microscope works well; however, metallurgical microscopes with superlong-working-distance objectives (such as the Nikon SLWD100X) are ideal. With 15× eyepieces and a 100× objective, there is enough magnification to allow direct observation of changes in the electrode tip as it is heated even for a final tip diameter less than 1 μm.

3. The polishing (heating) is done with a filament of 0.003-in. platinum–iridium (AM Systems) bent into a fine hairpin loop. The loop is coated with electrode glass to prevent the platinum from vaporizing onto the tip of the electrode being polished. The glass coating can be done by substantially overheating the platinum loop and then moving the tip of a pulled patch electrode directly against the platinum wire. The tip melts and begins to coat the

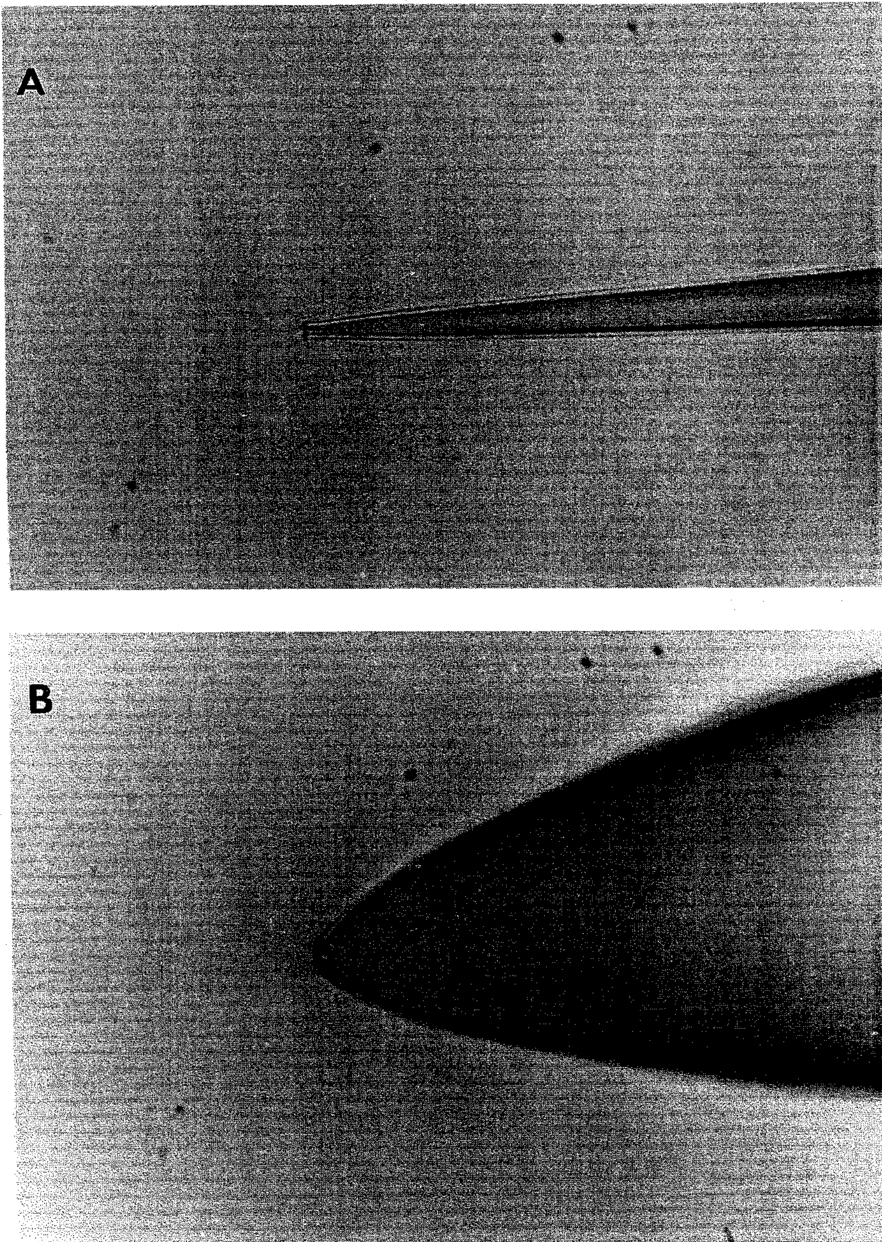

FIGURE 1 Photomicrographs showing the tip shape for (A) patch electrodes and (B) whole-cell electrodes.

wire and the electrode glass is then withdrawn while the wire is still hot. Three or four electrode tips may be required to fully coat this wire loop and to make a ball of glass encompassing it.

4. In firepolishing, the glass-coated wire is heated and brought close to the electrode tip so that the tip becomes rounded and smooth with an opening of 2–3 μm for whole-cell recording and 0.2–1 μm for single-channel measurements (Fig. 1).

D. ELECTRODE FILLING

1. Fill the electrode tip by simply dipping it into the desired filling solution. Alternatively, a suction apparatus made of a 10-ml syringe and a piece of tubing

can be used to draw up enough solution to fill most of the electrode shank. One to thirty seconds of suction is required depending on the size of the tip.

2. Insert a hypodermic needle of the right gauge and about $1\frac{1}{2}$ in. long into the back of the electrode and inject filling solution to the desired level (usually enough so it will just contact the tip of the reference electrode which will be placed into it later).

3. A bubble will remain behind the filled tip and in front of the back-filled fluid. Remove this by gently tapping against the electrode just above its shank. Verify by direct observation that all the bubbles are gone.

4. Using a hypodermic needle connected to a suction line, carefully remove any excess fluid from the pipette and vacuum any droplets away from the back of the electrode.

5. Place the electrode into the holder and verify that the reference electrode tip contacts the filling solution.

E. NOISE TESTING (PRIMARILY FOR SINGLE-CHANNEL RECORDING)

1. Using the manipulator, place the filled electrode in its holder just above the chamber in which the cells and their bathing solution are located.

2. Most modern patch-clamps contain an RMS noise meter. Read the value of the meter and be sure it is not more than 30% higher than the value of the headstage alone without its holder.

3. If it is, put in the holder alone. The noise increment should not be greater than 10–20% above that of the headstage alone. Greater increments usually mean that the holder is either dirty or wet. Dry it by blowing clean air or nitrogen through the holder suction line. If noise is still elevated, dismantle the holder and sonicate it first in distilled H_2O and then in ethanol. Dry for at least 3 hr at 60°C.

4. If noise is okay, proceed.

F. MAKING A SEAL

1. With the patch-clamp amplifier in voltage-clamp mode, immerse the electrode tip in the bath.

2. Adjust the pipette offset so that the current is zeroed.

3. Apply a 5-mV or so rectangular pulse and observe the resulting current pulse.

4. Lower the electrode gently against the cell until the current pulse falls to about 50% of its original value.

5. Using a 10-ml syringe with the barrel already pulled back to 7 ml, gently apply suction. Moving the barrel to the 8- to 9-ml position will usually provide enough suction for seal formation (Fig. 2).

6. If the seal does not form, remove the syringe, push the barrel all the way in, reconnect it, and then move the barrel back ever so gently. Often the seal will form with this reapplication of suction. You now have a cell-attached patch.

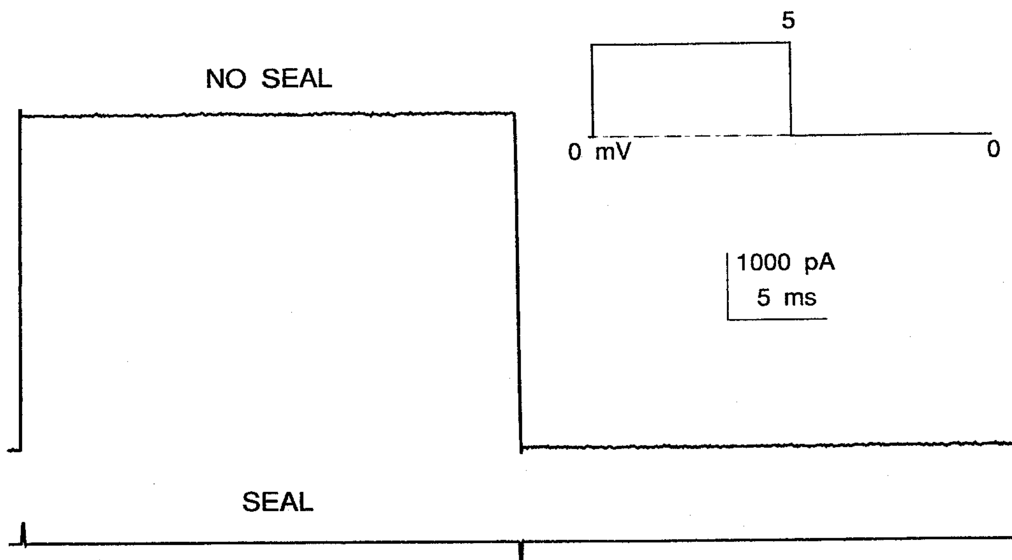

FIGURE 2 Current recordings before and after seal formation.

G. RECORDING CONFIGURATIONS (see Hamill, *et al.,* 1981)

1. Cell-Attached Patch

If there is a channel in the patch, you can go ahead and record in this cell-attached patch configuration. Realize, however, that the inside of the membrane is at the cell's resting potential and you can control only the potential in the pipette. Therefore, the transmembrane potential of the patch is usually unknown.

2. Inside-Out Patch

1. After sealing, rapidly move the electrode back from the cell surface. Usually a patch of membrane will be torn from the cell and remain adherent to the pipette tip.

2. Often a complete vesicle forms rather than just a patch. Single-channel currents in the vesicle are low in amplitude, may have rounded edges, or may droop in amplitude rather than being rectangular current pulses.

3. One side of the vesicle may often be disrupted by lifting the electrode tip into the air for 1–20 sec and then reimmersing it in the bath. Vesicles form less often and are broken more easily if a low-calcium bathing solution is used. An inside-out patch is so named because the inside surface of the membrane faces the bathing solution.

3. Standard Whole Cell

1. Shortly after seal formation while still applying the rectangular pulse, remove the suction syringe, push the barrel all the way in, reattach the suction line, and begin to gently withdraw the syringe barrel. Usually before you get to 1 ml, the patch of membrane will rupture and connect your pipette filling solution

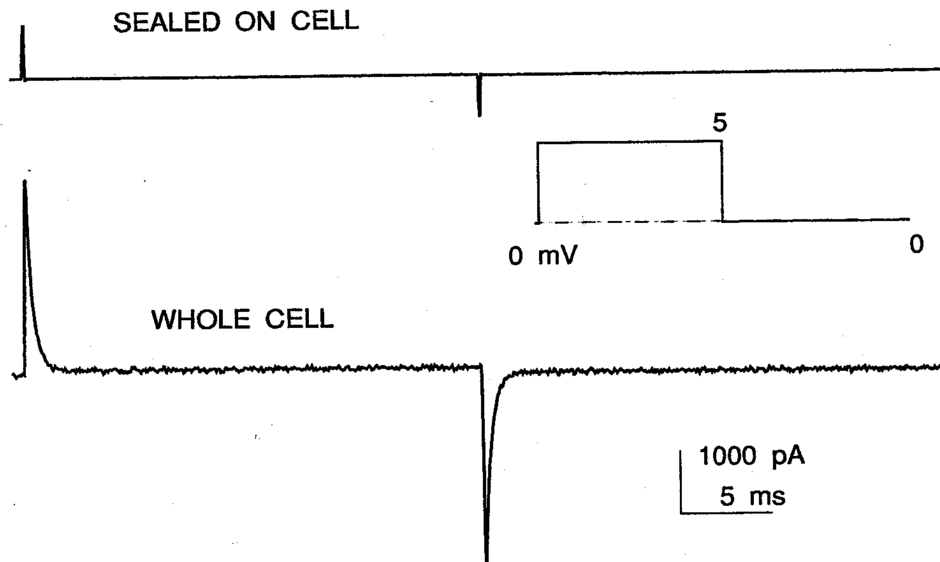

FIGURE 3 Current recordings before and after going whole cell.

to the cell interior. The current response to the voltage pulse changes as the whole-cell configuration is achieved, as shown in Fig. 3.

2. In some cells, it is very hard to disrupt the patch and a 20- or 50-ml syringe used as described above may be required.

4. Outside-Out Patch

After going whole cell by disrupting the membrane patch, slowly remove the pipette from the cell. Usually a stalk of membrane can be seen stretching between the pipette tip and the cell. When this stalk ruptures, an outside-out patch often forms. This configuration is so named because the outside surface of the membrane patch faces the bathing solution.

5. Perforated Patch Whole-Cell Recording (see Rae *et al.,* 1991)

1. Preweigh 3 mg of amphotericin B (Sigma A-4888) or Nystatin (Sigma N-3503) in 1.5-ml centrifuge tubes and store in the freezer.

2. At time of use, dissolve the antibiotic in 50 μl of DMSO and mix by pipetting in and out several times and then mix with a Vortex mixer.

3. Add 10 μl of this solution to 3 ml of the desired filling solution in a 15-ml screw-capped centrifuge tube. Shake vigorously and then vortex.

4. Draw 1 ml of the solution into a 1-ml syringe to use for back-filling electrodes.

5. Dip the tip of the electrode into the same filling solution without antibiotic and allow the tip to fill no more than 300–400 μl, then back-fill with the antibiotic solution in the usual way.

6. Following seal formation, the antibiotic will slowly diffuse to the tip and partition into the membrane patch. Partitioning forms channels permeable to Na, K, and Cl but not Ca^{2+}, Mg^{2+}, or glucose.

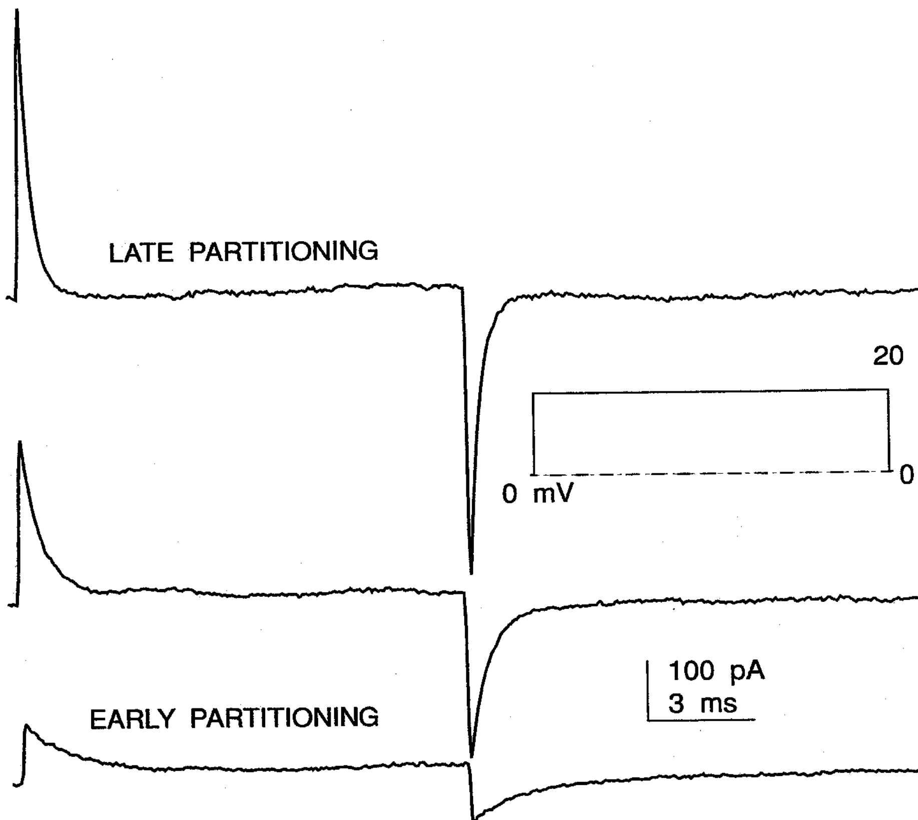

FIGURE 4 Current records of going whole cell with amphotericin perforation.

7. As partitioning occurs, the current response to a rectangular voltage pulse changes as shown in Fig. 4.

6. Perforated Outside-Out Vesicle (see **Levitan and Kramer, 1990**)

1. Once the antibiotic has produced an access resistance of less than 100 MΩ, simply remove the pipette from the cell.

2. This often results in a tiny vesicle at the tip whose pipette side is perforated with antibiotic. This is the functional equivalent of an outside-out patch but may also contain trapped cytoplasm and cell organelles. It can be used for reasonable signal-to-noise single-channel recordings.

H. RECORDING

1. Use one of the IBM-compatible or Macintosh computer systems and software which are commercially available. Alternatively, use a digital video tape recorder.

2. Be sure that the current output of the patch-clamp is filtered through a four- or eight-pole Bessel filter before going to either the computer or the tape recorder.

3. Verify that the digital sampling is at least three to five times the −3-dB bandwidth of the filter setting (Levis and Rae, 1992).

IV. Comments

For the highest-quality whole-cell recordings, the electrode resistance should be as low as possible (1–3 MΩ). For low-noise single-channel recordings, low-noise pipette glass and optimal elastomer coating must be used in combination with capacitive feedback electronics. Perforated patch whole-cell recordings are more physiological than standard whole-cell recordings and may prevent channel rundown.

V. Pitfalls

1. Proteins such as serum in the bathing medium often prevent seal formation.

2. Low-calcium filling solutions may make seal formation more difficult.

3. In perforated whole-cell recordings, the filling solution must contain the correct permeant anion concentration or the cell will be unable to control its volume (Rae *et al.*, 1991).

4. Improperly filtered and sampled current data can add unnecessary noise and obscure results.

5. Compounds that alter channel behavior may leach out of the electrode glass being used.

6. Recording of single channels in membrane patches may alter both channel kinetics and density.

7. It is very easy to record from membrane vesicles when you believe you are recording from membrane patches.

8. In standard whole-cell configuration, access resistance changes often occur during the recordings.

9. In perforated patch whole-cell recordings, many compounds placed in the electrode filling solution are unable to get into the cell.

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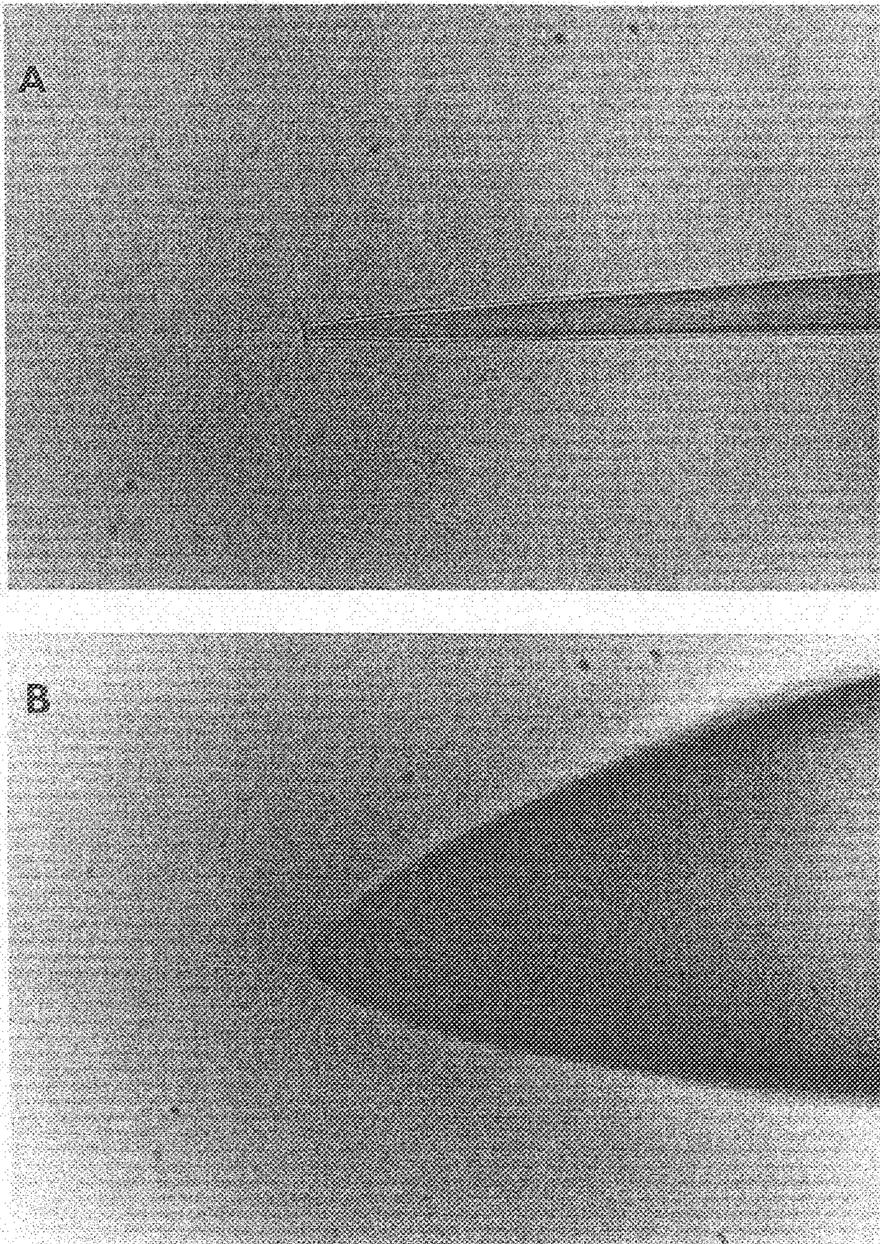

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