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Construction of Holding Pipettes for Microinjection

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Microinjection is a useful technique for introducing foreign genes into plant protoplasts[1-4] or cells.[5] These plant cells are often floating in a culture medium, and must be held securely to facilitate microinjection. The following describes the construction of pipettes used to hold protoplasts or cells.

Figure 1 shows a schematic of the device used for constructing the holding pipettes. It consisted of a nitrogen bomb for pressurization, a microforge (Narishige, MF-79) equipped with a pair of micromanipulators (not shown) and platinum heater; a binocular microscope and a variable autotransformer.

A glass capillary tube (Narishige, G-1) was first pulled using an electrode puller (Narishige, PG-1) to form a fine tip as shown in **Fig. 2** A and its other end was inserted into a silicone-tube connected to the regulator on the nitrogen bomb. The capillary was attached to one micromanipulator. A platinum wire or plate heater was held by the other micromanipulator. This heater was connected to an AC variable autotransformer (Yamabishi Electric, N-130-10).

The heater was then turned on by raising the output voltage of the autotransformer to 10 to 15 V. Then, under the binocular microscope (×120) the tip of the glass capillary was pressurized by nitrogen gas (0.5 to 1 kg/cm^2) and brought close to the surface of the heater. Upon heating, the end of the capillary was immediately sealed and the capillary became swollen near the point just below the sealed end to form a sphere as shown in **Fig. 2** B. The heater was then turned off and the tip was retracted from the surface of the heater. The size of the sphere can be controlled by changing the heater temperature and/or duration.

The sphere was removed from the micromanipulator and cut into a hemispherical shape (**Fig. 2** C) using a razor blade under the binocular microscope. The broken surface of the hemisphere was then fire-polished. (If necessary, the throat of the capillary can be constricted by the Hiramoto[6]). The finished pipette was then sterilized by autoclaving and used for microinjection[3].

Precleaning of the glass capillary either by blowing nitrogen gas or by washing with acid and filtered distilled water[7] is necessary to avoid clogging of the capillary with dust particles.

Another type of holding pipette (see **Fig. 2** D to F) can also be made, by a different technique, using the device shown in **Fig. 1**. A glass capillary was first pulled to from a blunt end (**Fig. 2** D) and this end was heated as described above to form a constricted capillary (**Fig. 2** E). **Figure 2** F shows an evacuolated protoplast of tobacco mesophyll held with this type of holding pipette.

The hemispherical holding pipette is more useful than the blunt-end pipette for holding intact (non-evacuolated) protoplasts because the presence of vacuoles render the protoplasts turgid and make it difficult to insert a glass micropipette into protoplasts unless they are held firmly in place. The hemispherical pipette has successfully been used for microinjection of DNA or other substances into protoplasts as reported previously.[3] On the other hand, the blunt-end pipette has been shown to be useful for microinjection of chromosomes into evacuolated protoplasts.[8]

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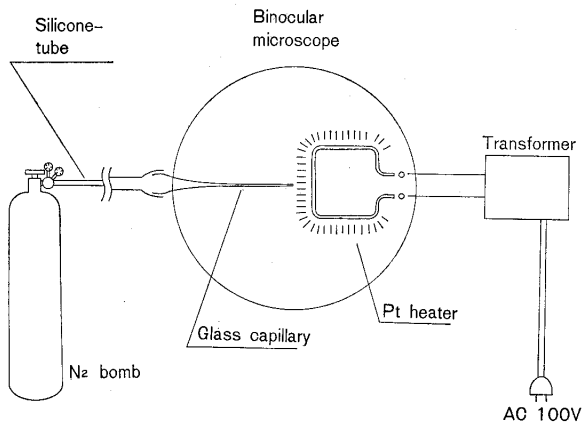

Fig. 1. A schematic of the device for processing glass capillary tubes to make "holding pipettes" for microinjection.

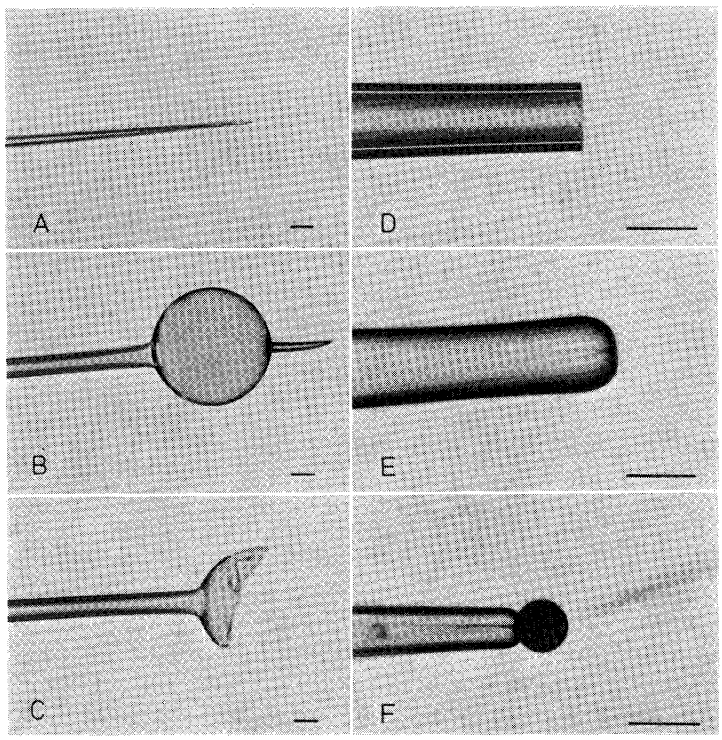

Fig. 2. Construction of a hemispherical (A to C) and constricted (D to F) holding pipette. To make a hemispherical pipette, a glass capillary was first pulled to form a fine tip (A). It was then heated under pressurization with nitrogen gas to from a sphere (B). This sphere was cut with a razor blade to a hemisphere (C). To construct a blunt-end pipette, a glass capillary was first pulled to form a blunt end (D) and its top was heated to make a constriction (E). An evacuolated-protoplast of tobacco mesophyll was held with a constricted pipette (F). Bar represents 30 μm.

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References

1) Morikawa, H. M., Y. Yamada, 1985. Plant Cell Physiol., **26**: 229-236.
2) Crossway, A., J. V. Oakes, J. M. Irvine, B. Ward, V. C. Knauf, C. K. Shewmaker, 1986. Mol. Gen. Genet., **202**: 179-185.
3) Reich, T. J., V. N. Iyer, B. Miki, 1986. Biotechnology, **4**: 1001-1004.
4) Nomura, K., A. Komamine, 1986. Plant Sci., **44**: 53-58.
5) Yamaoka, N., F. Yamamoto, I. Furusawa, M. Yamamoto, J. Shishiyama, 1987. Plant Tissue Cult. Lett., **4**: 66-70.
6) Hiramoto, Y., 1974. Exptl. Cell. Res., **87**: 403-406.
7) Okada, Y., A. Inouye, 1976. Biophys. Struct. Mech., **2**: 31-42.
8) Griesbach, R. J., 1983. Plant Mol. Biol. Rep, **1**: 32-37.

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マイクロインジェクションのための保持ピペットの作製

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植物細胞やプロトプラストにマイクロインジェクションするための保持ピペットの作製装置の概略および2つのタイプの保持ピペット作製法について述べた.