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Manual Pair Formation and Electrofusion of Protoplasts Using Platinum Microelectrodes

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Electrofusion using microelectrodes is a useful technique[1-3] for inducing cell fusion of pairs of plant protoplasts or animal cells and for following the fusion process thereafter. Here we report on the manual pair formation and electrofusion of protoplasts by using laboratory-made platinum microelectrodes with the aid of micromanipulators.

Figure 1 shows a diagram of the platinum microelectrode used in this study. A piece of fine platinum wire coated with silver (1 or 5 μm diameter platinum core, Japan Lamp Industry Co.) was soldered at one end to a copper wire (100 μm thick) which was inserted into a glass capillary (1 mm in outer diameter, Narishige, G-1). The other end of the copper wire was soldered to a lead wire. Both ends of the copper wire were fixed to the glass capillary with an epoxide resin (Ciba-Geigy, Araldite). The electrode was fixed to a plastic electrode holder (Narishige, H-1). In order to remove the silver coating, the tip of the microelectrodes was electrolyzed in 10% HNO_3 at about 7 V with Pt wire (3 mm in diameter) as a cathode, rinsed with distilled water and dried. Then an insulating varnish (General Electric Co. #7031) about 30% in concentration in a mixture of toluene and ethanol (1 : 1, v/v) was applied to the tip of the microelectrodes under a binocular microscope leaving 100 to 200 μm from the tip end uncoated. Microelectrodes were dried prior to sterilization with 70% ethanol.

A pair of the microelectrodes was attached to a pair of micromanipulators (Narishige, MO-104) which were placed[4] on an inverted microscope (Nikon, TMD) that rested on a vibration-free table in a laminar flow hood (Hitachi, PCHCS). The microelectrodes were connected to a pulse generator (Nikon Kouden, SEN 3102) via a booster amplifier (Nihon Kouden, 307 B)[3].

About 3 ml of a protoplast mixture containing 5×10^4 each of both protoplasts isolated[5,6] from cultured cells of *Coptis japonica* and *Euphorbia millii* in 0.6 M sorbitol with 2.5 mM $CaCl_2$ was placed in a plastic petri dish (60 mm in inner diameter) on the microscope stage. The microelectrodes were inserted into the liquid in the petri dish with the aid of the micromanipulators.

Heteroplasmic pairs of protoplasts were formed manually by gently pushing protoplasts to be fused with the tips of the microelectrodes as shown in **Fig. 2 A** and **B.** Immediately before pulsation, the tips of the microelectrodes were slightly taken back from the protoplast surface. For successful electrofusion it is essential that no direct contact is made between the tip of the microelectrodes and the protoplast surface during pulsation ; otherwise, the tip will stick to the surface on pulsation, causing lethal damage to the fused protoplasts.[3]

A single square pulse of 80 μA with a pulse width of 0.5 ms was then applied (the resistance between the microelectrodes in the present condition being 0.1 to 0.2 megaohm) and the protoplasts were fused into a spherical heterokaryon in 10 min after pulsation (**Fig. 2 C**). **Figure 2 D** shows three heterokaryons (indicated by arrows) which were manually paired and electrofused in the same manner as in **A** to **C**. They are with (top) or without (middle and bottom) dark-red vesicles which are known to form at vacuolar fusion by mixing vacuolar components (berberine and anthocyanin) as reported elsewhere.[3-6]

Figure 3 shows three typical configurations of the microelectrodes used for electrofusion. It was

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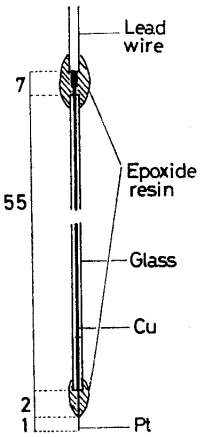

Fig. 1. Diagram of platinum microelectrode used in this study. The numbers represent the length of the parts in mm. See text for details.

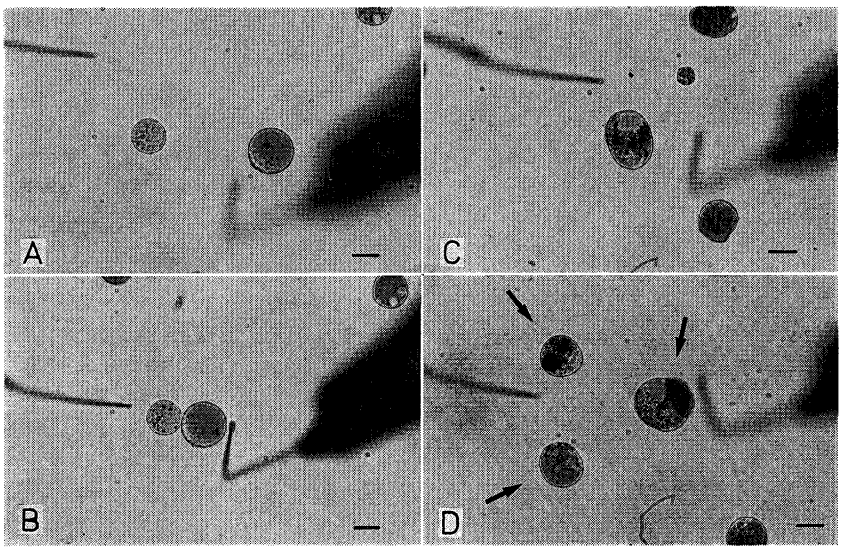

Fig. 2. Manual pair formation and electrofusion of protoplasts using platinum microelectrodes. (A) Before pair formation. *Coptis* (left) and *Euphorbia* (right) protoplasts are to be manually paired by use of microelectrodes (one being straight while the other bent); (B) After pair formation and immediately before pulsation; (C) Ten minutes after pulsation (80 μA with a pulse width of 0.5 ms); and (D) Three heterokaryons (indicated by arrows) produced as in (A) to (C). The bar represents 25 μm.

observed that configuration A required the weakest field strength to induce protoplast fusion while C required the strongest field strength for fusion was yet most efficient for manual pair formation; B was an intermediate in both respects.

Platinum microelectrodes are more useful than glass microelectrodes[1] because the former can be streilized and are reusable. Berg[2] used much thicker stainless steel or platinum microelectrodes (20 to 100 μm thick) for animal cell fusion. We also tried 5-μm platinum microelectrode but 1-μm

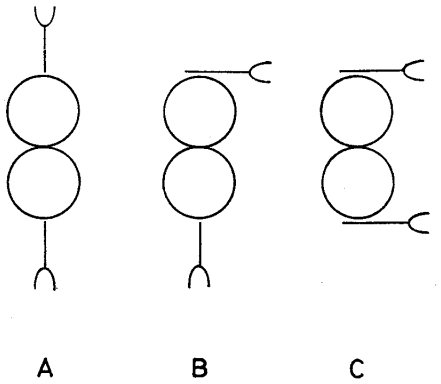

Fig. 3. Three typical configurations of microelectrodes for electrofusion.

microeletrodes gave better results in the plant protoplasts used in this study.

No cell fusion method so far reported can induce selective cell fusion or fusion between selected protoplasts apart from a sophisticated device reported by previous authors.[7] The present method is the only simple and direct method by which in principle, any protoplasts may be paired and fused by pulsation. Furthermore, we can follow details of the cell fusion process thereafter as reported elsewhere.[3,5]

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白金微小電極を用いた植物プロトプラストの対形成と融合

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植物プロトプラスト融合のための白金微小電極の作製法について述べた．顕微鏡下，この微小電極対をマイクロマニピュレーターで操作してプロトプラスト対を形成させ，電気パルスにより融合させた．電気融合における微小金属電極の利点について議論した．