Information Panel

Components of a Microinjection System

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INTRODUCTION

Direct-pressure microinjection with a micropipette is an essential tool for introducing a variety of impermeant substances into the cytoplasm or nucleus of plant and animal cells. Microinjection remains the most direct method to gain insight into the function and dynamics of intracellular components, to produce transgenic animals, or to overcome male infertility. This article describes the basic components of a microinjection system.

OVERVIEW

A basic microinjection system requires an inverted light microscope (Fig. 1, part 1), micromanipulator (Fig. 1, part 2), micropipette holder (Fig. 2, part 3), gas pressure regulator (Fig. 1, parts 3 and 4), micropipette puller, glass capillary tubing, micrometer syringe, and vibration isolation table (Fig. 1, part 9). More elaborate systems can be assembled according to the experimental needs of the investigator. For example, the system can be supplemented with a specimen incubator, CCD (charge-coupled device) camera (Fig. 1, part 5), shutter controller (Fig. 1, parts 6 and 7), epifluorescence (Fig. 1, part 8), digital image processing software, and computer.

COMPONENTS OF A MICROINJECTION SYSTEM

Microscope

Any sort of inverted microscope (Fig. 1, part 1) equipped with phase-contrast, Hoffman phase-contrast, or Nomarski optics (differential interference contrast [DIC]) can be used for microinjection. A 25× or 40× dry objective with high numerical aperture (NA) is typically used for microinjection of adherent cell cultures. The lower-magnification objective (25×) is used to find and guide the micropipette tip to the surface of the cell. However, this objective does not give sufficient magnification for microinjection where a 40× objective is used. A novice learning the technique can switch from one objective to another during capillary replacement and microinjection.

Depending on the size and/or geometry of the specimen, different optics may be preferred. If the specimen is flat (well-spread cells in culture), phase contrast works better than DIC. When cells are rounded (early stages of cell spreading, neuronal primary cell culture) and nuclear injection is required, DIC is preferable.

Some experiments require selecting cells for microinjection that are already expressing fluorescent fusion proteins. To visualize the fluorescent signal, the microscope should be equipped with a mercury lamp, shutter controller, neutral density filter set, and a single dye filter cube. A combination of fluorescence observation and microinjection requires planachromatic objectives (Plan-Fluor or Plan-Fuotar).

If the investigator wants to microinject and then immediately observe cells with high resolution, it is possible to inject using the 60× or 100× oil-immersion objective; however, with these objectives, it is more difficult to find the needle due to the narrower plane of focus.

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FIGURE 1. Microinjection system.

1. Nikon Diaphot inverted microscope. 1a, Microscope stage; 1b, shutter for transmission light.
2. Leitz micromanipulator. 2a, Headstage; 2b, coarse adjustment of the transverse movement; 2c, coarse adjustment of the sagittal movement; 2d, coarse, and 2e, fine vertical adjustment; 2f, joystick operator.
3. Narishige air pressure regulator, model IM-200.
4. Connections to compressed air from house supply.
5. Photometrics SenSys CCD camera.
6. UniBlitz shutter driver 1 model VMM-DI (for epifluorescence).
7. UniBlitz shutter driver 2 model SD-10 (for transmission light).
8. Power supply for mercury lamp.
9. Vibration tabletop on (9a) supports (TMC, Peabody).
10. Custom-made container for micropipettes.

Glass Capillary Tubing

Glass capillary tubing is used for micropipette fabrication. Glass tubing stock is available in different barrel and cross-section configurations and also with a wide range of diameters and wall thicknesses. Capillaries are offered in two configurations—with or without an inner filament. Capillaries with inner filaments can be easily back-loaded (see below) as the solution is drawn to the tip by capillarity. A thin-wall circular one-barrel glass capillary with an outer diameter of 1–1.5 mm is preferred for microinjection of mammalian cells. The outer diameter of the capillary and the thickness of its wall are two of the most important characteristics of glass tubing. For microinjection of small or sensitive cells, a narrower outer diameter gives a finer tip, which enables delivery of a smaller volume of injectant and helps minimize cell injury. The thickness of the tubing wall is also important because a pulled micropipette has the same ratio of outer/inner diameters at the tip as the original capillary tubing. Having a larger internal diameter for the pulled capillary improves flow characteristics and prevents frequent clogging. However, tips that have a large outer diameter will produce a large wound at the cell surface. A micropipette pulled from thin-wall tubing optimizes both the fineness of the tip and the size of its opening.

The most popular material for micropipette fabrication is borosilicate glass that provides excellent strength. For some special applications, soda glass or alumino-silicate glass tubing may be preferred. Quartz tubing is also available. However, due to its high melting point, quartz cannot be pulled using

FIGURE 2. Orientation of the micropipette to the microscope stage. A dish containing cells (1) is placed into the special adaptor on the microscope stage. The micropipette (2) is inserted into the instrument collar (3) attached to the headstage of the micromanipulator. The micropipette forms an angle of $\sim 30\text{--}40^\circ$ to the microscope stage. The micropipette tip is lowered into cell culture medium directly above the center of the lens.

conventional pullers. The Sutter laser-based P-2000 puller is ideal for this purpose (Sutter Instrument Company).

There is no special requirement for washing or treatment of glass capillary tubing prior to micropipette fabrication. However, if desired, micropipettes can be sterilized or silanized inside a multipurpose pipette storage container (e.g., World Precision Instruments [WPI] or similar). Pipettes are silanized by adding silane to the reagent well in the center of the container. A container with pipettes in situ can be dry-heat sterilized at 170°C . Of course, sterilization of micropipettes occurs when they are pulled (see below).

Pipette Puller

Micropipettes are fabricated with a micropipette puller. The main principle of pulling is application of tension from the ends of the glass tubing while the middle of the tubing is heated and melted by a heating filament. The heating filament has at least one loop (circular or squared) where the capillary tubing is centered. When the heating device is turned on, a current runs through the filament causing it to heat up, thereby melting the middle of the capillary tube. The ends of the capillary tube are clamped into two arms of the machine, which will electronically or gravitationally pull apart, producing two micropipettes.

A pulled micropipette consists of four parts: the tip, the shank, the shoulder, and the shaft. The shape (length and angle of the shank) and diameter of the tip opening are dependent on the current, tension of pulling, or length of gravity fall (in the case of a vertical puller). By adjusting values for the various parameters in a pulling sequence, a wide variety of pipette shapes can be achieved.

A factor that can adversely affect pipette shape is residual heat in the heating element. A platinum alloy filament helps to eliminate this problem as it has an extremely low thermal mass and cools very rapidly. The tip of the pipette pulled below the heater element is not affected by residual heat.

Numerous types of micropipette pullers are available—from very simple mechanical to sophisticated models fashioned with electronic programming. Either horizontal or vertical pullers are also available.

David Kopf Instruments is at the top of the list of companies producing pipette pullers. Other suppliers include MicroData Instrument, Energy Beam Sciences, HEKA Instruments, WPI, Stoelting, and GENEQ.

Micromanipulator

A micromanipulator is an essential part of a microinjection system (Fig. 1, part 2). It is used to hold and manipulate a micropipette during microinjection. The micromanipulator consists of two main parts: headstage (Fig. 1, part 2a) and remote controller (Fig. 1, parts 2b–2f). The headstage holds and moves the micropipette, and the controller regulates pipette movement. The remote controller usually includes both coarse (Fig. 1, parts 2b–2d) and fine (Fig. 1, part 2e) control adjustments and a dynamic joystick operator (Fig. 1, part 2f). The position of the micropipette tip can be moved in four ways: horizontal movement (sagittal [X] and transverse [Y]), vertical movement (Z), and tilt angle (T). When adherent cells are microinjected, the micropipette is held at an angle of 30°–40° to the microscope stage (Fig. 2). During microinjection, the tip of the micropipette penetrates the cell by vertical (z axis) movement.

Micromanipulators can consist of two separate units (e.g., Narishige) or be designed as one piece of equipment (e.g., Leitz, WPI). To ensure stable drift-free micromanipulation, Leitz and WPI bolt the headstage to a solid massive base with strong magnetic feet. The base also serves to elevate the manipulator to the level of the microscope stage. The joystick arm and control knobs are located at the base. When a micromanipulator includes two separate units (headstage and controller), the headstage is attached directly to the microscope stage (a special adapter is required) or maintained on the magnetic stand. The remote controller should be placed on a vibration isolation table at a comfortable distance for hand manipulation.

Macromanipulators have a variety of driver mechanisms: mechanical (e.g., Leitz, Leica), oil or water hydraulic (e.g., Narishige, Newport Corporation), and stepper motor (e.g., Zeiss, Eppendorf, WPI). Models such as the Eppendorf InjectMan NI2 (Eppendorf) and motorized micromanipulator SM325 (WPI) have programmable joystick units. Each of these micromanipulators has attributes that are designed to ensure smooth and drift-free movements of the micropipette. A shorter step of the movement provides finer micromanipulation.

A micropipette holder (instrument collar; Fig. 2, part 3) is required for attaching the micropipette to the headstage. Micropipette holders are sold separately from the micromanipulator and are available in many different sizes. The size of the holder should match the outer diameter of capillary tubing to hold the micropipette tightly.

Air Pressure Regulator

An air pressure regulator is used to adjust the pressure of compressed gas that forces the outflow of a small volume of liquid from the micropipette tip. We use the Narishige IM-200 air regulator (Narishige USA) (Fig. 1, part 3). It controls both the pressure and the duration of the injection. Depending on the pressure applied, a volume ranging from microliters to femtoliters can be delivered. Practically, it is possible to microinject a constant volume within a 50% difference among cells. For a method of measuring the injection volume based on the fluorescence intensity, see Wang et al. (1982).

Pressure is applied pneumatically from either a tank of nitrogen or with compressed air from the house supply (Fig. 1, part 4). Compressed air can be used only for injection of oxygen-insensitive materials, whereas nitrogen has general application. The regulator gives a digital readout of the applied pressure in psi/kPascal. The duration of injection is adjusted using an internal clock with a digital switch.

The Narishige regulator has three auxiliary features: fill/hold, balance, and clear. The fill/hold feature is used to generate suction to fill a micropipette from the tip or to hold a cell with a second pipette. A low balance pressure is applied to generate slow flow from the tip between injections, preventing pipette clogging and inflow of the medium into the pipette. The clear feature generates a momentary application of high pressure to clear a clogged tip.

Vibration-Free Environment

A vibration-free environment is required for successful microinjection. To prevent uncontrolled mechanical displacements of a micropipette tip and injected specimen, the system should be mounted on a

vibration isolation table (Fig. 1, parts 9 and 9a). The table consists of a heavy tabletop (Fig. 1, part 9) on supports (Fig. 1, part 9a) that can be inflated by compressed air. When the supports are inflated, the table rides on a cushion of air that damps floor vibrations. Newport Corporation and Technical Manufacturing Corporation (TMC) are two companies that provide vibration isolation tables.

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