Electrochemical Attosyringe: Application to Solution Injection into Living Cells

by François Laforge

The possibility of manipulating ultra-small volumes of solution (injection in living cells and vesicles, microfluidics, capillary chromatography, nanolithography, etc.) is of a considerable practical and fundamental interest.[1-4] In life sciences, it is often necessary to inject a material of high molecular weight (DNA, proteins) directly into cells because their membranes are impermeable to suchmolecules.[5]

Our electrochemical attosyringe consists of a nanopipette produced by heat pulling a glass capillary, filled with an organic solvent (e.g., 1,2-dichloroethane) and immersed into aqueous solution. When a suitable voltage is applied between the internal and external reference electrodes (Fig. 1), the surface tension at the liquid/liquid interface changes. The force that develops at the three-phase boundary (water-organic-glass) results in the ingress or egress of the solution. Figure 2 shows a reversible ingress/egress sequence of solution. The amount of the dispensed liquid can be controlled through the voltage sequence applied to the device.

The attosyringe was used to inject ethidium bromide (EB) fluorescent dye into live mammalian cells. A 200-nm pipette was attached to the SECM, which was used to position it a few microns away from a cell (Fig. 3a), and then to penetrate the cell. By applying a suitable voltage sequence, a controlled volume of sucrose buffer containing 10 µM EB was injected inside cells 2, 3, and 4. In a control experiment, the same pipette was brought inside cell 1, but the solution was not injected. Figure 3b shows a fluorescence image of the same field of cells taken after having rinsed the Petri dish several times with a buffer solution containing no EB. The injected cells (cells 2, 3, and 4) fluoresce, while other cells—including the control cell 1—do not because EB cannot cross a mammalian cell membrane.[6]

The electrochemical attosyringe was shown to be a useful device for volume-controlled microinjection of material into living mammalian cells. ■

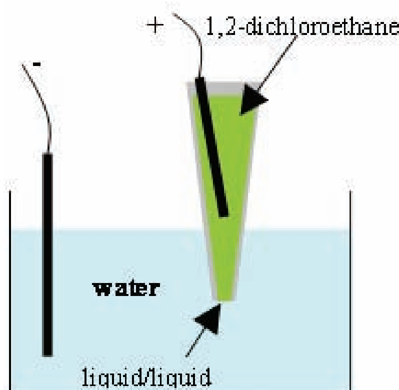

FIG. 1. Scheme of the electrochemical attosyringe arrangement.

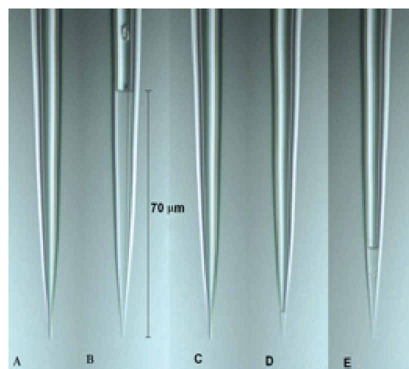

***Fig. 2.** Sequential ingress/egress of water in a 1,2-dichloroethane-filled nanopipette (a) initial immersion, E = +600 mV; (b) ingress of water after the potential was stepped to -100 mV and then to +90 mV; (c) complete egress of water at E = +600 mV; (d) same as (b) but with a shorter step time at E = -100 mV; (e) the potential was stepped again to -100 mV and then back to +90 mV. The tip-radius was 300 nm.*

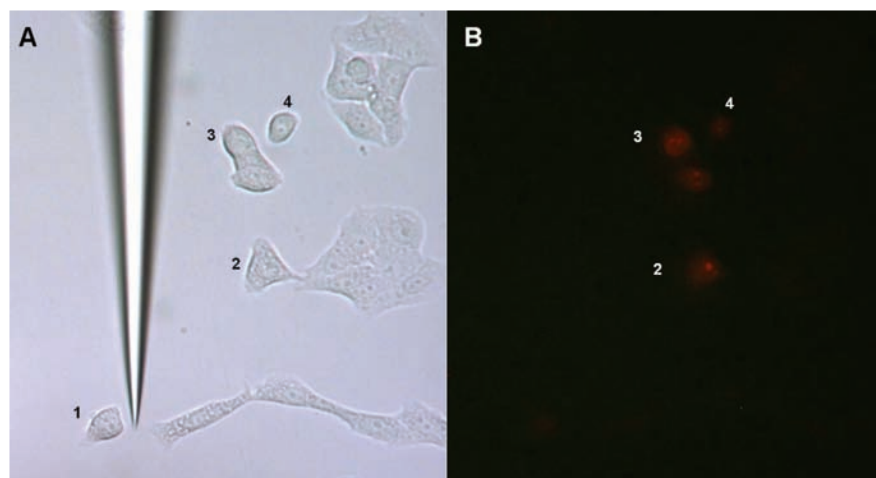

***FIG. 3.** Optical (a) and fluorescence (b) images of a cell field. Cells 2, 3 and 4 were injected with a 10 µM ethidium bromide buffer solution. Cell 1 was penetrated by the nanopipette without dye injected.*

References

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