Versatile Piezoelectric Driver for Cell Puncture

Michael Fromm, Paul Weskamp, and Ulrich Hegel
Institut für Klinische Physiologie, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45

Abstract. A simple and versatile tool facilitating micropuncture of small cells is described which utilizes a commercial piezoelectric element made from a stacked column of monomorph ceramic discs. The device is able to advance complete input stage-electrode-assemblies with high speed and can be used in combination with conventional micromanipulators. Advancing characteristics as recorded optically at high magnification demonstrated less axial vibration, although faster action, than two other modern micropositioners driven by step motors. In biological experiments on selected tissues (Necturus gallbladder epithelium, Amphiuma renal distal tubule cells, rabbit and human corneal endothelium) the combined use of micromanipulator and piezo-stepper was, in all cases, superior to the use of a micromanipulator alone: the percentage of successful cell penetrations increased, cell potentials were stable for a longer time, and the durability of electrode-tips improved.

Key words: Micropuncture technique — Epithelial cells — Intracellular impalement.

Introduction

The puncturing of single living cells with microtools without either reducing cell viability or impairing physiological function has presented considerable technical problems. Numerous devices have been designed for this purpose. Most of such devices utilize either step motors [2], or piezoelectric bender elements [3-8, 10], or tubular ceramic piezoelectric elements [6] as drivers. However, up to now, some experimenters have rejected such electromechanical puncture aids because of too much unpredictable movement of all existing systems (cf. [9]). They prefer to rely on the simple method of advancing the microelectrode tip until dimpling of the cell membrane occurs and then tapping slightly on the micromanipulator or its base.

In this paper, it will be shown that it is easily possible to construct a micropuncture aid of good performance by choosing a commercially available piezoelectric element made from stacked ceramic discs. We will present both direct recordings of the mechanical action and first experiences from biological experiments in some selected epithelial cell types and also provide a comparison of the described piezo-stepper with other commercially available systems.

Materials and Methods

The core of the system was a piezoelectric driver element (type P-172, Physik-Instrumente, Waldbronn, FRG), which consists essentially of a column of 36 polarized circular ceramic discs. The discs are glued together by epoxy, connected electrically in parallel and mounted in a rectangular metal housing (42 x 15 x 15 mm). The front disc bears a short steel piston with a thread inside, which allows mounting of any kind of capillary- or microelectrode-holder. The electrical capacitance of these elements is about 40 - 50 nF, the maximum working voltage 1 kV. At this voltage, the piston advances more than 20 μm. The safe mechanical load exceeds 100 kg.

The piezoelectric element was fitted into a massive block of lead weighing 1 kg with outside dimensions of 80 x 40 x 40 mm. This mass takes up the repulsion which occurs at sudden acceleration of the electrode-holder. In our design, the holders plus input stages weighed 35 or 80 g, respectively, for one or two input stages of an electrometer-amplifier (model F 223-A, WP-Instruments, New Haven, CT, USA). The latter were utilized when bearing double-barreled electrodes. The whole system was mounted on a standard micromanipulator (Leitz, Wetzlar, FRG), as shown in Fig. 1.

The very simple electric circuit is shown in Fig. 2. Since piezoelectric crystals can be represented electrically — with some simplification 1 — by a single capacitor C_p, the forward and backward speed could be varied by adjusting resistors R_1 and R_2, respectively, the electrical forward and backward time constants then being R_1 C_p and R_2 C_p, respectively.

1 For discussion of the complete equivalent-circuit, see, e.g. [1]
Fig. 1. The piezo-stepper, mounted between micromanipulator and electrometer input-stages. 1 Standard micro-manipulator Leitz. 2 Lead block, containing piezo-element P-172, mounted on standard ball-and-socked joint Leitz. 3 Coaxial high voltage cable, connected to piezo-element via submin coaxial connector. 4 Holder (aluminium), bearing two WPI F223-A input stages which hold a double-barreled micro-electrode.

Step width was preselected, or position could be changed by adjusting the output voltage of the high voltage power supply (Keithley Instruments, Cleveland, OH, USA). Addition of a second voltage supply connected to $R_2$ would offer the possibility of pre-selecting any step size from any starting position. A complete equipment with this feature will be manufactured by Physik-Instrumente, D-7517 Waldbronn, FRG. A 40 µm element will be optional. Preliminary experiments have shown that, with respect to the fast reaction of the piezo-element, $S_2$ has to be absolutely free of chatter. Therefore, a remote-controlled ($S_1$) mercury-contact relay was used for $S_2$. The actual position of the piezo-stepper could be monitored via a 1:1,000 voltage-divider ($R_3$, $R_4$), which provided a suitable output voltage for a chart recorder. The lead block and the housing of the piezo-element were kept on ground potential. With this, electric shielding of the system proved to be sufficient, since cross talk between a 1,000 V step of the driving voltage ($R_1C_p$ set to 100 µs) and the microelectrode output (tip resistance 10 MΩ) was only about 1 mV without any special screening of the microelectrode and its Ag/AgCl/KCl-connector.

At monitoring the electrometer amplifier output it was observed, that, however, the rapid acceleration of the input probe induced an output signal much greater than this small and also very short electric cross talk. Oscillations of up to 150 mVpp, decaying within 2–3 ms were observed with our F 223-A input probes.

A direct recording of absolute speed and of mechanical vibrations of microelectrodes advanced by the piezo-stepper and, for comparison, by either one of two step motor driven devices was accomplished by use of a microscope (Orthoplan, Obj. 25 ×, Leitz, Wetzlar, FRG), equipped with a scanning slit and a photomultiplier recording system. A tiny flag of black film was glued onto the broken-off end of a microelectrode 5 cm long, and the holder adjusted so that movements of the flag were within the field of the scanning slit. The output signal of the photomultiplier, which was linearly correlated with the mechanical feed of the electrode, was recorded on a storage oscilloscope (type 5103 N, Tektronix, Beaverton, OR, USA). The advancing distance of all three devices was adjusted to 25 µm. The speed of the two step motors was set to maximum, while the electrical time constant of the piezo-stepper was adjusted to $\tau = 0.5$ ms. The slave-cylinder of the hydraulic microdrive and the piezo-stepper, respectively, were mounted on the Leitz manipulator, whereas the nanostepper was fixed onto its own pertinent manipulator. In a fourth condition, the input stage with microelectrode was fixed directly onto the Leitz manipulator and the effect of slightly tapping on the manipulator was recorded. In all these experiments concerning

2 At monitoring the electrometer amplifier output it was observed, that, however, the rapid acceleration of the input probe induced an output signal much greater than this small and also very short electric cross talk. Oscillations of up to 150 mVpp, decaying within 2–3 ms were observed with our F 223-A input probes.