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Encapsulated Petri dish system for single-cell drug delivery and long-term time lapse microscopy

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Abstract

We have developed a system that allows focal drug application for cell culture microscopy. Single-cell drug delivery is achieved through the insertion of a patch-clamp-like micropipette in a microenvironment-controlled chamber mounted on a standard 35-mm Petri dish. The system has precise control of temperature, CO_2 concentration, and humidity, while preventing contamination during experiments. The use of standard Petri dishes allows long-term experiments by alternating in situ microscopy with incubator growth. Modern biological long-term experiments such as the characterization of drug effects on cell movement, axonal guidance, mitosis, apoptosis, differentiation, or volume regulation can be performed. The chamber is compatible with any inverted microscope without significant modifications.

Keywords: Culture chamber; Long term; Time-lapse microscopy; Real time; Drug delivery; Single-cell manipulation

Time-lapse video recording of cell populations has become a powerful tool in modern biological research. This technique allows the researcher to observe the behavior of the system under study in long-term experiments involving cell migration [1,2], axon guidance [3–6], and cell differentiation [7,8]. Experiments involving manipulation of cell cycle or cell death also benefit from long-term monitoring and time-lapse recording. Experiments involving drug application require mid- to long-term follow-up of the cells under treatment, as the effects of the chemicals can vary at different times [9]. During long time-lapse experiments, cell cultures should be kept under the microscope and homeostatic conditions maintained for hours or even days.

Three basic microscopy culture systems have been developed: open, closed, and box chambers. All systems provide the basic environmental requirements for cell growth over extended periods. These requirements include: sterility, stabilized pH, osmolarity, and temperature [10–12]. Open chambers allow external manipulation and are generally used in experiments lasting only a few hours. Environmental variables are barely controlled, or not at all controlled, in open systems [10]. On the other hand, closed chambers are sealed off from the environment to ensure the health of the cultures, and are used for experiments that can last many days [13–17]. However, their closed architecture largely restricts cell manipulation. Box-type chamber incubators consist of a transparent plastic box in which the microscope, or part of the microscope, is enclosed [18,19]. Although manipulation is easier, the system suffers from the problems of all open systems: difficult environmental control and the potential risk of contamination. In addition, these systems also consume large quantities of CO_2, and the microscope and/or the camera must be protected from the highly humid and somewhat acid atmosphere inside the box.

This work introduces an encapsulated Petri dish (EPD)[1] system useful for mid- and long-term time lapse video microscopy. Designed for standard 35-mm Petri dishes,

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[1] *Abbreviations used:* EPD, encapsulated Petri dish; $[Ru(bpy)_3]Cl_2$, ruthenium (II) tris(2,2′-bipyridyl) chloride; STS, staurosporine; AVD, apoptotic volume decrease.

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the EPD system is suitable for use with any inverted microscope. It allows the insertion and precise positioning of a micropipette to conduct physiological manipulation of targeted cells in the culture. Long-term cell viability tests using the BHK-21 cell line are described, as is complete time-lapse microscopy of a culture during staurosporine-induced cell shrinkage [20], using a time- and space-controlled micropipette.

Materials and methods

Encapsulated Petri dish

The EPD system is composed of three main elements, illustrated in Fig. 1A: A polycarbonate cover (1) seals the dish, forming the encapsulated chamber; the bottom half of the Petri dish itself (2) fits inside an aluminum heater (3), where the micropipet manipulator is attached.

To perform a typical experiment, a cell culture in a standard 35-mm Petri dish was taken from the incubator to the flow hood, and its cap was replaced with a polycarbonate cover, which fits tightly on the Petri dish with a silicone ring. An indium–tin oxide glass window (CEC120S, Präzisions Glas & Optik, Iserlohn, Germany) was used as a resistance heater (80 mA) to prevent condensation, thereby avoiding artifacts resulting from the presence of drops in the microscope light pathway. Glass micropipettes were made from filament capillaries (No. 615000, A-M Systems, Carlsborg, WA, USA) pulled with a vertical pipette puller (DKI 700C, David Kopf Instruments, Tunjunga, CA, USA) and inserted into the chamber through a small hole in a coverslip-sized Mylar film. This film covers a 10×7-mm square hole in the polycarbonate body, and was able to freely slide on this surface to follow micropipette movement. This allows for several millimeters of X–Y displacement of the micropipet, while keeping the chamber

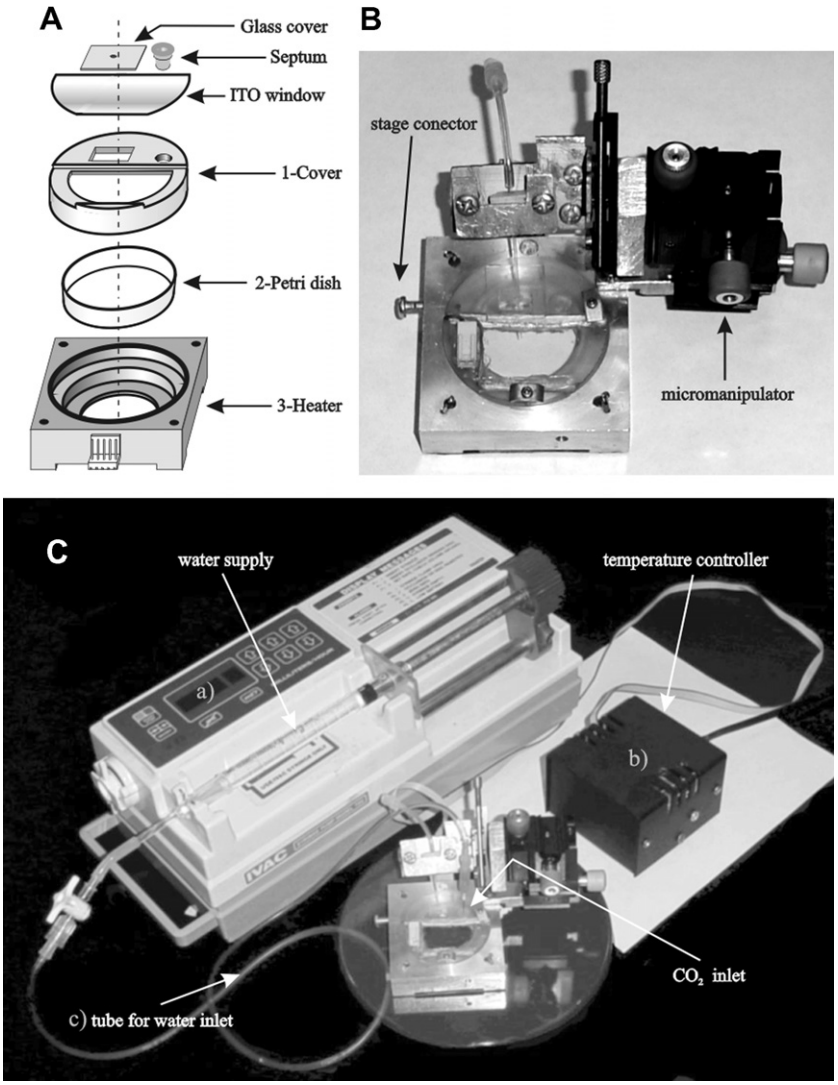

Fig. 1. Encapsulated Petri dish (EPD) system. (A) Exploded view of the main parts of the EPD. (B) Assembled EPD, top view. Heater block with micromanipulator, Petri dish, and inserted micropipette are visible. (C) Overall view of the EPD system, its water supply (a), and its temperature controller (b). Water and CO_2 are carried through silicone tubing (c) and enter the EPD chamber through needles puncturing the septum.