ON-CHIP NON-INVASIVE VOLTAGE CLAMP ON XENOPUS OOCYTES

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ABSTRACT

In order to improve the throughput of the traditional "Two Electrode Voltage Clamp" (TEVC) measurements on *Xenopus* oocytes, we present here a chip-based non-invasive technique for the measurement of ion channels activity. We focus especially on the integration of the electrical measurement site into a microfluidic structure, allowing rapid exchange of various small-volume solutions.

Keywords : Microfluidics, Single cell analysis, Voltage clamp, *Xenopus* oocyte.

1. INTRODUCTION

Regulated ion exchange between the cell cytoplasm and its external environment, through ion channels in the impermeable lipid membrane, is a vital function of all living cells. In order to study the impact of a possible new drug candidate on a specific ion channel, *Xenopus laevis* oocytes, expressing exogenous ion channels, are often used as host cells for electrophysiological investigations. In the TEVC technique, the oocyte is impaled by two glass microelectrodes (Figure 1a) [1]. However, this requires individual and time consuming cell handling with micromanipulation under a microscope.

2. NON-INVASIVE SYSTEM

In our planar chip-system shown in Figure 1b, the cell is immobilized by suction on a conical hole (ca. Ø 250 µm).

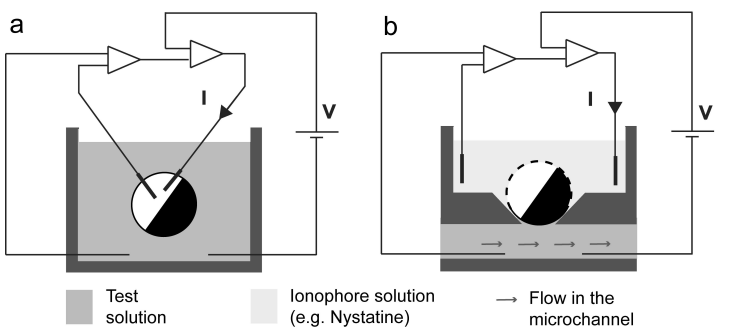

Figure 1. Schematic view of the voltage clamp system a) the traditional "Two Electrode Voltage Clamp" (TEVC) setup, b) our non-invasive chip-based configuration.

Similar approaches for the immobilization of cells have been investigated by our group for small cells in a patch-clamp system [2] and by others for oocytes in an impedance analysis system [3].
The idea of the present project is to obtain a low contact resistance electrical access to the cell interior by permeabilizing the upper part of the cell membrane by means of an ionophore (e.g. nystatine) [4], whereas the lower part remains untouched. No puncturing with microelectrodes is required, as illustrated in Figure 1b. A four-point measurement with two Ag/AgCl electrodes, on both sides of the hole in the chip, allows recording the ion current through the lower part of the cell membrane under voltage clamp conditions. In order to obtain a good signal-to-noise ratio, a tight fluidic and electric isolation between the upper and lower compartments has to be achieved. This seal is established by the portion of the cell membrane which is in direct contact with the side-walls of the suction hole.
For evaluating the reaction time of the ion channels to different chemical agents, rapid fluidic exchange on a time scale down to 100 ms is to be integrated into this system, thanks to a microfluidic channel structure under the cell (Figure 1b). The flow in the two input channels can be adjusted to rapid shifting of the fluidic interface under the cell as shown on the simulation in Figure 2.

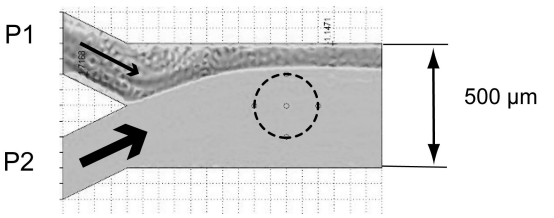

Figure 2. Simulation of the hydrodynamic behavior of two laminar fluid flows merging in a single channel. The fluidic interface may be shifted by adjusting input pressures P_1 and P_2 (here $P_1 < P_2$). The cell position is indicated by a dashed line.

3. FABRICATION

The conical hole and the microfluidic channel underneath are molded in poly-dimethylsiloxane (PDMS). The PDMS is irreversibly bonded to a structured glass substrate, with fluidic access. Figure 3 shows a photograph of the microchip.

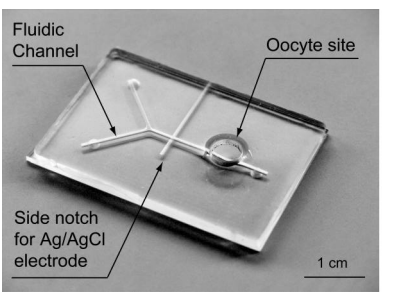

Figure 3. Photograph of the oocyte voltage clamp microchip, consisting of a top layer in PDMS bonded on a glass substrate.

4. RESULTS AND DISCUSSION

Promising results on *Xenopus laevis* oocytes have been obtained with the first tested prototypes. Fluidic leakages could be minimized between the two parts of the system and electrical isolation gives us a "seal" resistance of about 60 kΩ. This was sufficient to measure sodium amiloride-sensitive currents in oocytes expressing the human Epithelial Sodium Channel (hENaC) and to obtain amiloride-sensitive I-V curves (Figure 4) with a large signal-to-noise ratio. The big advantage of nystatine is its reversible effect. Even after exposure to this ionophore, the cell membrane can recover its initial state. As a result, the measurements were first performed on the microchip and cells have been re-used on the standard TEVC setup. Measurements obtained with the two methods compare well.

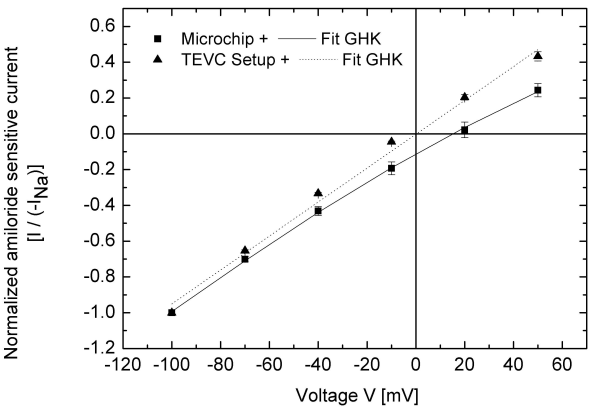

Figure 4. Current-Voltage curves obtained with the two measurement methods (microchip and TEVC). The amiloride sensitive current is normalized using the current value $-I_{Na}$ at -100 mV. The fits are based on the "Goldman Hodgkin Katz" (GHK) current equation [5].

5. CONCLUSION

We present a new chip-based system for electrophysiological measurements on *Xenopus* oocytes. We could demonstrate that measurements of ion currents through the cell membrane with our new system compare very well with those obtained with the traditional TEVC technique. Our low cost system needs less handling and uses a simple fabrication process. Ongoing work focuses on the integration of a rapid exchange fluidic system, in order to study kinetic reactions of ion channels.

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