A MICROFLUIDIC DEVICE FOR AUTOMATED ELECTROPHYSIOLOGICAL MEASUREMENTS ON XENOPUS OOCYTES UNDER ZERO GRAVITY

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ABSTRACT

A non-invasive electrophysiology system for performing rapid measurements on Xenopus laevis oocytes under varying gravity levels is presented. Our novel approach makes the need for inserting microelectrodes into the cytosol unnecessary. Immobilization of the oocyte on top of an orifice is achieved using a slight air overpressure to ensure that the cell is kept in a stable condition even under varying gravity levels.

In our present studies, we overexpressed the epithelial sodium channel (ENaC) and the sodium/phosphate cotransporter (NaPi-IIb). Results obtained under laboratory conditions as well as under microgravity and hypergravity demonstrate the system’s robustness and give first indications of ENaC’s susceptibility to changes in gravity.

KEYWORDS: electrophysiology, non-invasive, Xenopus laevis, epithelial sodium channel, sodium phosphate cotransporter, gravity

INTRODUCTION

Unsolved medical problems related to the exposure of the human body to an extended period of time to microgravity are still jeopardizing future manned missions. A major issue in this perspective is the muscle and bone atrophy that forces astronauts to exercise for about 2.5 hours daily to compensate. To understand the biochemical mechanisms underlying this physiological phenomenon, a microfluidic device for voltage clamping Xenopus laevis oocytes under zero gravity was developed. It consists of a multi-layer design employing precision machining for the oocyte immobilization site and PDMS micromolding technology for microperfusion. The device allows studies on ion transport mechanisms of specific channels under varying gravitational environments. In contrast to standard methods like the two-electrode voltage clamp (TEVC), the device presented here, which we term asymmetrical voltage clamp (ATOVC), employs a non-invasive methodology - the cell does not need to be impaled by the electrodes [1][2]. Moreover, as the immobilization of the oocyte does not rely on the Earth’s gravitational field, this device works under varying gravity conditions.

THEORY

The principle of the ATOVC is based on the separation of the cell’s membrane surface into two discrete areas. For Xenopus laevis oocytes, this has first been demonstrated in the transoocyte voltage clamp (TOVC) [3]. This method involves clamping across the whole oocyte with an AC voltage to determine changes in total capacitance. Control of the membrane potential, however, is not possible in this method. The ATOVC, on the other hand, effectively separates the total capacitance and resistance of the cell into two lumped impedances: a large (body) membrane impedance and a small (patch) membrane impedance akin to a loose macropatch configuration [4]. Because of the high resistance ratio (~40) the voltage drop across the body membrane is minimal compared to the voltage drop across the patch membrane (Fig. 1).

Figure 1: LEFT: (a) Equivalent electronic circuit of the ATOVC setup. (b) Membrane area distribution as a function of orifice diameter. (c) Voltage distribution as a function of orifice diameter. RIGHT: Cross-sectional view of the microfluidic core module.
Any change in measured transoocyte conductance will be predominantly dependent on change in conductance of the patch membrane. The patch membrane can be superfused inside a microfluidic channel with activating and deactivating solutions to modulate the conductance of ion channels and transporters. By subtracting data obtained from a perfusion sequence involving buffer solution and a modulating solution, currents sensitive to the particular agent can be obtained. This implies that the leak conductance, a result of the uneven membrane topology, remains constant throughout the experiment. A similar system, described earlier lacked precise control of the leak conductance [2].

EXPERIMENTAL

![Microfluidic core module and Complete ATOVC system](image)

*Figure 2: Left: Microfluidic core module. Right: Complete ATOVC system ready for parabolic flights.*

The microfluidic core module houses the upper compartment for the oocyte, the microperfusion system and the electrodes for the voltage clamp (Fig 2., left). To gain access to the upper compartment the top sealing layer is removed. Then, the oocyte is pipetted into the upper compartment, after which the top sealing layer is remounted and its screws tightened to allow for an airtight seal. The upper compartment is then pressurized using a computer-controlled pressure regulator. After allowing the oocyte settle for 5 minutes, the perfusion system is activated. This comprises another electronic pressure regulator and three computer-controlled solenoid valves (Fig. 2., right). The ATOVC system is then ready for the perfusion sequence, which can be programmed in the proprietary user interface connected to the microcontroller.

Since the ATOVC system was developed specifically for use under microgravity conditions, a number of features not provided by laboratory-based electrophysiology systems were implemented. The entire fluidics system is closed, starting from the pressurized solution vials and ending with a special inflatable waste container. The use of a microfluidic channel is vital to the system to allow experiments being conducted during extremely short periods of time, as is the case in parabolic flight maneuvers.

RESULTS AND DISCUSSION

First, a number of experiments under laboratory conditions were performed to optimize the ATOVC system with regards to its stability against fluctuations in pressure differentials, which is crucial for guaranteeing a constant leak conductance. The optimal immobilization pressure was determined to be 20 mbar. Time-dependent experiments performed on oocytes overexpressing the epithelial sodium channel (ENaC) demonstrate the system’s stability and sensitivity (Fig. 3a). Moreover, the channel blocking response (10 µM of amiloride) was very fast thanks to the laminar flow around the patch membrane and fast switching of the solutions. Experiments conducted on oocytes overexpressing the (flounder) sodium/phosphate cotransporter (NaPi-IIb) demonstrate that the ATOVC can also be employed for measurements on carrier proteins. Also, the use of ionophores on the body membrane of NaPi-IIb overexpressing oocytes resulted in an improvement in the voltage control of the patch membrane to give a voltage-dependence comparable to conventional electrophysiological recordings, but scaled according to the patch area (Fig. 3b).

Studies onboard an aircraft flying parabolic maneuvers were performed to study the influence of gravity on the activity of ENaC. It has been shown before that ENaC’s conductance is sensitive to external mechanical stimulation [5]. Comparison of I-V traces of the amiloride-sensitive current reveal that the conductance of ENaC is reduced at 0 g (Fig. 3c, red trace). In contrast, studies conducted in a hypercentrifuge show that ENaC’s conductance is increased when exposed to a gravity level of 1.8. In this case, the amiloride-sensitive current at -50 mV transoocyte voltage was recorded over the course of 30 minutes.
CONCLUSION

A system for performing non-invasive electrophysiological measurements on *Xenopus laevis* oocytes under varying gravity levels was realized. Experiments conducted in the laboratory demonstrate that the ATOVC system is able to faithfully reproduce the results typically obtained by the TEVC method. Furthermore, due to the high stability of the leak conductance, transoocyte current measurements with high sensitivity can be performed. Results from ENaC overexpressing oocytes exposed to micro- and hypergravity suggest that ENaC activity is indeed sensitive to gravitational forces. Future experiments on other ion channel classes under micro- or hypergravity will provide additional biologically relevant insights, into which physiological pathways are affected by gravitational change. The main advantages of this system, in agreement with our design criteria, are: (i) the minimal time needed for setup and measurement, (ii) capability of fully automatic operation (after mounting the oocyte) and (iii) high reliability. Even though the system was developed specifically for electrophysiological studies under varying gravity level, due to its operational simplicity it could also be conveniently used for non-invasive electrophysiological investigations in ground-based laboratories.

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REFERENCES


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