A BRIEFCASE-SIZED SYSTEM FOR TOXIN DETECTION USING PLANAR PATCH CLAMP

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ABSTRACT

Toxint'patch is a unique transportable system designed to detect and quantify microalgae neurotoxins in seawater samples or shellfish flesh using planar patch clamp. It is meant to be used in coastal laboratories, for directly on-site monitoring of the seawater and shellfish quality.

We present here all the elements integrated recently to make the briefcase. We also explain the last developments performed on the system to improve its reliability and automate the analysis procedure.

Current efforts are put in the validation of this system for the detection of saxitoxin, a PSP (Paralytic Shellfish Poisoning) phycotoxin that targets voltage gated sodium channels.

KEYWORDS: Planar Patch Clamp, Paralytic Shellfish Poisoning, Toxin Detection, Automation

INTRODUCTION

The toxins produced by some species of microalgae, represent a major health and economic issue . Their detection is today fulfilled using either the semi-quantitative mouse bioassay which is controversial for both ethical and scientific reasons and analytical methods like ELISA immunoassay or LC-MS that require either specific training or expensive and non transportable equipment.

Our system is based on the MultiPatch planar patch clamp system , an electrophysiology technique that measures the ion currents through the cell membrane. In fact, some of these toxins target ion channels, thus modifying their electrical behaviour. Such 'electrical signature' of the toxin interaction addresses the need to provide new specific and sensitive tools for adequate warning and monitoring purposes.

The MultiPatch system was recently validated with some of these toxins on voltage-gated ion channels, in particular with gonyautoxin and HEK (Human Embryonic Kidney) cells expressing sodium channels Nav1.5 using a commercial electronic device (Figure 1). The performances of the silicon chips were also optimized -. Starting from this system, we developed a briefcase sized patch-clamp system, Toxint'Patch, designed to allow a non-expert user to perform parallelized patch clamp recordings using recombinant cellular sensors. This compact system allows directly on-site analysis, within coastal analytical laboratories. This system is intended to offer an automated, accurate and user-friendly solution to detect and quantify the presence of phycotoxin in seawater samples or shellfish flesh to prevent poisoning.

The application is currently focused on the detection of gonyautoxin, a member of paralytic shellfish poisoning (PSP) phycotoxines that specifically inhibit sodium channel currents hNav1.5 (Figure 1.B).

INTEGRATION OF THE BRIEFCASE-SIZED SYSTEM

The new briefcase device (Figure 2) had to reach two goals: combine all the elements of the planar patch-clamp system in the briefcase and allow automated measures.

Therefore, a new pneumatic assembly system which forms the heart of the system was designed with KeyOx (Gillonay, France) and installed in the briefcase to hold all the elements together (the chip, the silicone rings and the PCB layers that form the planar electrodes). The o-ring shaped gaskets were replaced by silicone plates moulded with the 9 o-ring shaped holes for the 9 sites (Figure 4) and the holding is now performed with suction cups and vacuum (Figure 3 & Figure 4), thus reducing the setting time.

To control the system, a specific electronic circuit was developed with Easii-IC (Grenoble, France) and integrated in the device. It includes the assembly system for planar patch clamp lock control, the solenoid valves and pressure regulators for the fluid supply, as well as the signal measuring part. Each module was validated separately.

The whole system is driven by a specific interface also developed with Easii-IC (Figure 5). This software was designed to allow the user a complete flexibility of use.

Finally, to allow automated recording procedure, all the steps have to be automated, especially the filling procedure. This is done with the computer interface which can be adjusted for optimization.

RESULTS AND DISCUSSION

The recording part of the circuit was successfully tested and validated using regular patch clamp techniques (Figure 1.A). We used HEK cells expressing $hNa_V1.5$ ion channels in suspension in a cell bath solution (composition in mM: 137 NaCl, 4 KCl, 5 $BaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH = 7.3 with 6M NaOh) and an electrophysiological medium in the micropipette that matched the ionic composition of the cytoplasm for whole-cell recording (composition in mM: 135 CsF, 10 CsCl, 5 NaCl, 5 EGTA, 10 HEPES, pH = 7.3 with 5M CsOH). This part was also successfully validated with a model cell that reproduces the equivalent electronic circuit of the patch clamp experiment directly within the case (Figure 6).

The efficiency of the fluidic assembly system and the silicone plates design were validated through pressure resistance tests in the lower chamber. These tests allowed proving that the system was watertight even at maximum pressure (2 bar). This module has also been validated, using commercial Axon electronics.

Since all the elements are integrated within the briefcase, they have to be validated together, starting with the automation of the filling procedure which is currently in progress. The filling of the lower chamber is quite reproducible but the whole step can only be validated with a good electrical measure of the chip resistance which would certify that the filling was successful.

CONCLUSION

After the validation of both fluidic and electronic parts, the measurement procedure has to be automated. Electrical control allows checking and validating each step of the procedure thanks to resistance and capacitance measures.

The next steps consist in improving the reproducibility of the filling step, getting measures of the chip resistance within the assembly system filled but without cells, and then improving the rate and quality of the sealing of HEK cells on the chip.

Once the system is validated on gonyautoxin, it can be used with other cells and targets to detect other kinds of toxins that also disturb ion channels currents.

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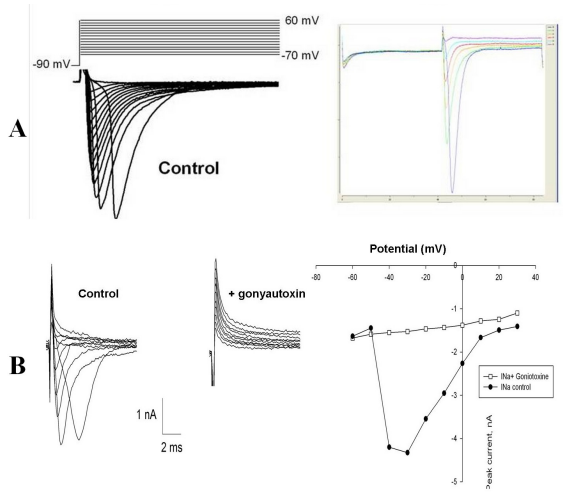

Figure 1: sodium channel currents recording
A: conventional patch clamp with commercial electronics (left) and Easii-IC electronic card (right).
B: planar patch clamp using commercial electronics. I/V curve without and with specific inhibitor (Gonyautoxin)

Figure 2: general view of the briefcase.
Electronic card is placed at the bottom stage and fluidics with pneumatic assembly of chip, at the upper stage.

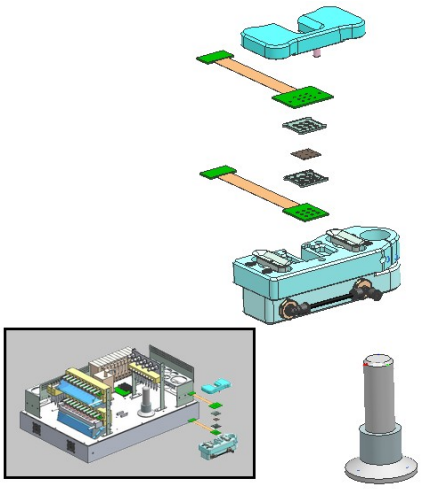

Figure 3: exploded view of the pneumatic assembly of the system

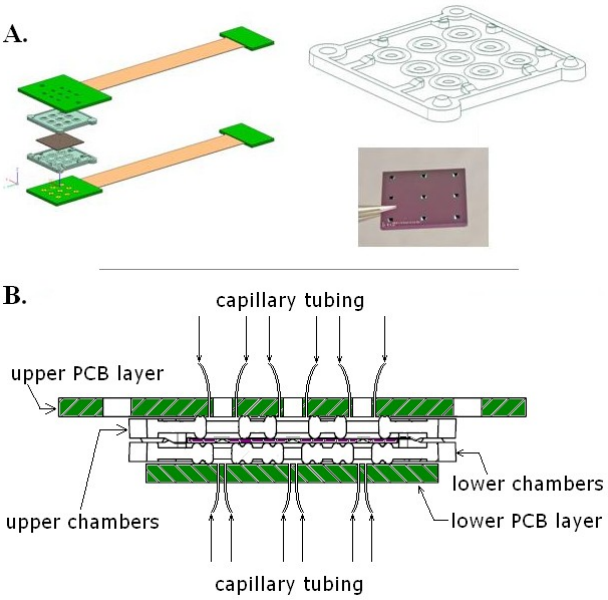

Figure 4:A. schematic view of the assembly system. From top to bottom : upper PCB layer, upper ring layer, silicon chip, lower ring layer, lower PCB layer
B. sectional view of the assembled system

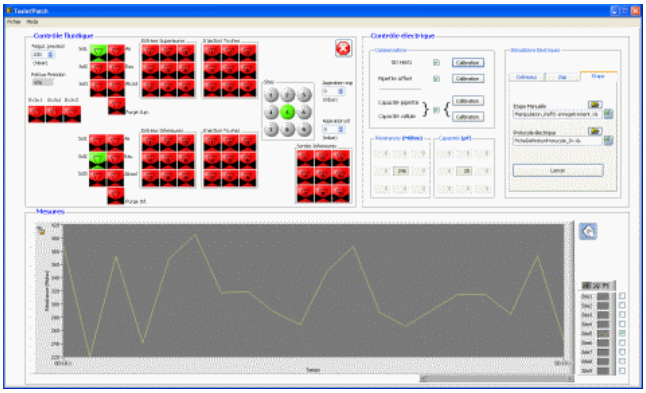

Figure 5: manual mode of the man/machine interface:
- *Solenoid valves control buttons (upper-left corner)*
- *Evolution of the resistance value measured (bottom)*
- *Measurement settings (upper right corner)*

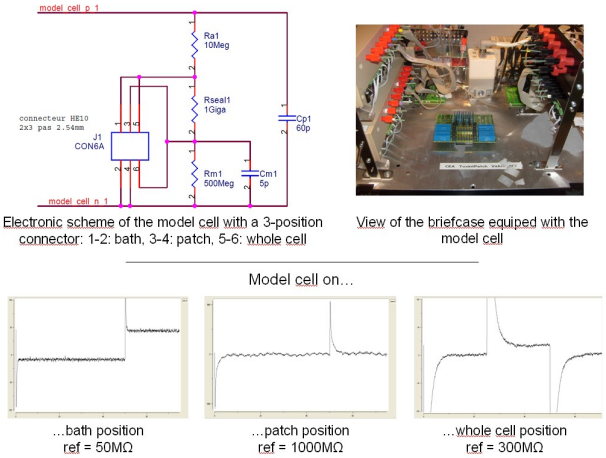

Figure 6: model cell validation of the recording electronics