A Missing Factor in Chip-Based Patch Clamp Assay: Gigaseal

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Abstract. The “gold” standard in the study of ionic currents across biological membranes is the Patch Clamp method. However, this is a slow, labor and skill intensive process. High throughput patch clamp devices are mainly chip-based. A major challenge in these miniaturized devices is the low rate of “Gigaseal” formation which is critical in the study of Single Channel effect. In a conventional patch clamp, a pipette moves and patches a fixed cell (cell-adhered patch) which is grown on the bottom of a Petri dish. In the chip-based case, the cells are in suspension and move towards the fixed patch clamp sites (cell-suspended patch). In this study, using the proven conventional patch clamp setup, we investigated the effect of the differences in the cell configurations between the convention patch clamp and cell-based patch clamp. It is shown that adhered cells (as used in the conventional setup) have a much higher rate of gigaseal formation as compared to the cells in suspension (as used in chip-based devices). We postulate that the arrangement of the cytoskeleton within the cell plays a major part in the formation of the gigaseal.

Keywords: Patch Clamp; Microfluidics

1. Introduction
Ion channels are proteins residing across the cell membrane that act as gates to the interior of cells. They are involved in rapid signaling, electrical excitability and fluid transportation throughout the body. Hence ion channels are significant targets in the drug discovery process.

The “gold” standard in studying ionic currents is the patch clamp method\(^1\). The patch clamp technique involves moving a micropipette by a micromanipulator to prod a cell, trapping a portion of the cell and then forming the various patch clamp configurations for ionic currents measurements. Although this technique is slow, labor-intensive and demands a skilled operator, it is useful for drug
screening. However, the demand of high throughput analysis for today’s drug discovery process makes the conventional patch clamp unsuitable. The pursuit of creating a high throughput patch clamp is mainly focused on chip-based device. These are microstructures fabricated on substrates like glass, silicon with treated or coated surfaces or microstructures molded using poly(dimethylsiloxane) (PDMS)\textsuperscript{2-4}. These devices are mostly planar except for two which have a lateral architecture\textsuperscript{2,4} (Fig. 1).

Fig. 1. Schematic diagrams of (A) a planar and (B) lateral patch clamp device\textsuperscript{4}.

A challenge encountered in prior attempts is the lack of or low percentage of gigaseal formation\textsuperscript{2-4}. This is a condition where the cell membrane and the walls of the patch clamp pipette form a tight seal creating a high resistance path for current flow between the extracellular environment and the pipette. This low noise condition is critical for the study of single channel in the membrane. Although, the low gigaseal formation rate can be attributed to the chip material, aperture geometry and surface roughness, the main difference in the configuration between the conventional and chip-based patch clamp has been overlooked. In the conventional patch clamp, the cells are fixed to the bottom and a pipette moves and patches a fixed cell (cell-adhered patch). In the chip-based case, the cells are in suspension and move towards the fixed openings (cell-suspended patch). Thus, it is beneficial to study the effect of suspension of cells on the rate of gigaseal formation. To address this, we assumed that conventional patch pipette has high gigaseal occurrence. Hence, by using a conventional patch clamp, we can investigate the effect of the differences between cell-adhered and cell-suspended patches. The result of this investigation may shed some light on the gigaseal formation and help in modifying the designs of chip-based patch clamp devices to attain high rate of gigaseal formation.

2. Materials and Methods

2.1. Cell culture

PC12 cells used were cultured in a similar way as previously reported\textsuperscript{4}. A coverslip containing attached cells were transferred to a fresh 35 mm dish containing 2 ml of external solution for patch clamp. Another coverslip was trypsinized and spun down at 1000 rpm at 4°C for 5 minutes. The cells were collected then resuspended in the external bath solution for patch clamp.

2.1.1. Electrophysiology

The external bath solution contained (in mM): 150 NaCl, 2.8 KCl, 10 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 10 HEPES and 2 mg/ml glucose pH 7.2 (310 mOsm). The internal solution for patch pipettes contained (in mM): 130 K-gluconate, 2 NaCl, 20 HEPES, 4 MgCl\textsubscript{2}, 6H\textsubscript{2}O, 4 Na\textsubscript{2}ATP, 2.5H\textsubscript{2}O, 0.4 NaGTP and 1 EGTA. The recording amplifier used was a HEKA EPC9 amplifier with its headstage mounted on a hydraulic micromanipulator.

Patch clamp experiments on adhered and suspended PC12 cells were performed using separate pipettes pulled and polished from the same glass capillary (Fig. 2). The initial access resistances of the two pipettes (R\textsubscript{pip}) are similar, with an average difference of 1.5MΩ and the diameter of the internal pipette tip opening is about 1–2μm.
The pipettes were filled with approximately 6μl of internal solution using a syringe. A positive pressure of about 3-5 kPa was applied to the pipette before the pipette was lowered into the solution to prevent contamination of the pipette tip. Suction was applied once the tip touches the cell for the cell-adhered case or when the tip is about 100μm away from the cell in the cell-suspended case. The applied pressure in both cases was about 23kPa. Suction was held until a gigaseal was formed or until five minutes had passed if no gigaseal were formed. The negative pressure was then released to atmosphere for five minutes before the patch clamp attempt was discarded. The holding time of five minutes in both cases was chosen based on experience derived from the conventional patch clamp process. Initial resistance ($R_{pip}$) and maximum resistances reached during the suction as well as after the suction were recorded ($R_{max}$).

3. Results and Discussions
A total of 20 experiments were performed for both cell-suspended and cell-adhered cases. A representative data set is plotted in Fig. 3.

A comparison is made between the data obtained for the adhered patch and the suspended patch ($n = 20$) using SPSS software. The difference in the maximum sealing level achieved for the different cell configuration is found to be significant ($P>0.99$).

Several other interesting observations can be made from the investigation. The gigaseal configuration attained in the cell-adhered mode is achieved in a very short time. The release of suction in the cell-suspended mode causes the seal resistance to drop (Fig. 4). This is consistent with the experience from the conventional patch clamp of adhered cell that release of suction before gigaseal will impede or reverse the sealing process.
Maximum resistance reached during patching

Fig 3. Plot of maximum resistance attained for cell-adhered and cell-suspended patches

Maximum resistance reached during suction and after suction release (cell-suspended mode)

Fig. 4. Plot of maximum resistance attained for cell-suspended mode during suction and after suction release

Most importantly, the mean level of maximum seal achieved for suspended cell case is $107.5 \pm 42.2\,\text{M}\Omega$. The maximum seal reached is $213\,\text{M}\Omega$. The low mean and maximum seal values obtained with patching cells in suspended are incidentally consistent with most of reported chip-based patch clamp devices’ performance.
4. Conclusion
The effect on the rate of gigaseal formation for cell in suspended (as in chip-based patch clamp devices) was investigated and compared to the adhered cell (as in conventional patch clamp setup) using a proven conventional patch clamp platform. The result clearly shows that cells in suspension has low success rate of gigaseal. The low success rate is even more evident when compared the adhered cell patches. This difference in the success rate is statistically significant. Several literatures on conventional patch clamp have reported that a cell membrane condition is very critical in a successful gigaseal formation. Cells change their structures during spreading and rounding. The force generation and internal balance between cell cytoskeleton and extracellular matrix changes the cell internal structure and thus changing the membrane’s mechanical property. Based on the experimental results, we postulate that the local tension induced in the adhered cell membrane when the tip is pressed on it and the “stretched” state of the membrane in cell-attached mode may have played a significant role in the gigaseal formation.

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