Chapter 6

**Studying mechanosensitive ion channels with automated patch clamp: comparison of conventional and automated patch clamp systems**

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**Abstract**

Patch clamp electrophysiology is the main technique to study mechanosensitive ion channels (MSCs), however conventional patch clamp is laborious and success and output depends on the skills of the operator. Even though automated patch systems solve these problems for other ion channels, they could not be applied to MSCs. Here, we report on activation and single channel analysis of a bacterial mechanosensitive ion channel, using an automated patch clamp system. With the automated system, we could patch not only giant unilamellar proteo-liposomes but also giant *Escherichia coli* (*E. coli*) spheroplasts. The tension sensitivity and channel kinetics data obtained in the automated system are in good agreement with that obtained from conventional patch clamp. The findings will pave the way to high throughput fundamental and drug screening studies on mechanosensitive ion channels.

Part of this chapter has been published in *Eur Biophys J.* (43, 97-104 in 2014)

The experiments presented in figures 3A, 4A, 4B and 4C have been performed by me plus the cloning, purification of MscL and preparation of spheroplasts, whereas the data shown in figures 3D, 3E, 3F, 4D, 4E and 4F are from the work of Maria Barthmes and data from figures 3B is from the work of Jan-Peter Birkner.
INTRODUCTION

Mechanosensitive ion channels (MSCs) are molecular transducers of mechanical force in species from all kingdoms of life. They are involved in physiological processes ranging from touch and hearing, to blood pressure control (1). The best-characterized MSCs are located in the inner membrane of bacteria and protect the bacterial cell from lysis upon a sudden osmotic downshift (2). Under these conditions, water rushes into the cells, the cell turgor increases; this in turn increases the tension in the membrane. MSCs sense the membrane tension directly, open a pore and allow the passage of ions and even some small molecules. Bacterial MSCs for example MscL is one of the most studied and fairly understood among the MSC found in nature. Understanding how they function will therefore provide invaluable insights for understanding the eukaryotic mechanosensation (3).

The most important tool to investigate MSCs has been the patch clamp electrophysiology method (4). In patch clamp, application of a gentle suction to the patch pipette draws the membrane of interest onto the tip of the pipette and promotes a tight gigaohmic sealing between the membrane and the pipette (5). After the seal formation, the suction is released. To activate MSCs, the sealed membrane is stretched by applying negative pressure to the patch pipette (6). It has been possible to study MSCs by patch clamp using giant spheroplasts from E. coli (7), giant blisters, which can be obtained from collapsed multilamellar liposomes (8), and using giant unilamellar liposomes with reconstituted ion channels (9). In spite of invaluable data obtained from conventional patch clamp experiments, the major challenges have been the requirement for a thorough training, reproducible fabrication of patch pipettes, the labor-intensive nature of the system, and the limited number of successful patches per day (10).

The advances in automated patch clamp made it possible to study many other ion channels routinely and with high throughput (10), but so far it has not been possible to study MSC channels by such automated systems. In this chapter, we explain a method on an automated planar patch clamp system (Port-a-patch) to study one of the best characterized MSCs: mechanosensitive channel of large conductance (MscL) from E. coli both in its native environment in giant E. coli spheroplasts and in its reconstituted form in giant unilamellar liposomes (GUVs). We measured the tension sensitivity and single channel characteristics of MscL by both automated and conventional patch clamp and show that mechanosensitive channels can be studied by automated patch clamp.

MATERIALS AND METHODS

Expression and Purification of MscL.

MscL was expressed and purified as explained before (19). Briefly, E. coli strain, PB104 (20) containing the vector with the mscL gene was used. A single colony was inoculated into a 10 ml Luria-Bertani (LB) medium plus 100 μg/ml ampicillin and 10 μg/ml chloramphenicol during the day. An overnight culture (50 ml) was prepared from the day culture (1:100 dilution). Cells were then grown in 2L fermenters (1: 40 dilution) using LB medium supplemented with 100 μg/ml ampicillin and 10 μg/ml chloramphenicol. When cells reached an A600 of about 2.5, protein expression was induced by addition of arabinose (0.1% v/v). After 2 more hours of growth, cells were harvested by centrifugation and rinsed using 25 mM Tris-HCl (pH 8). The harvested cells were disrupted using a Constant cell disrupter (Type TS/40; Constant Systems) set to 25,000psi. The resulting cellular debris was removed by a low spin centrifugation step (18,460 x g for 30 minutes at 4°C). The supernatant was then centrifuged at high speed (145,400 x g for 90 minutes at 4°C). The resulting vesicles were homogenized in 25 mM Tris-HCl buffer, pH 8 to a final concentration of 0.7 g/ml and subsequently frozen in liquid nitrogen and stored at -80°C until needed. The vesicles were solubilized in detergent buffer (1% Triton X-100, 300 mM NaCl, 50 mM sodium phosphate, 35 mM imidazole, pH 8). The insoluble material was removed by centrifugation (267,000 x g for 20 minutes at 4°C). The solubilized material was added to a previously equilibrated nickel NTA matrix for binding and incubated at 4°C for 30 minutes with gentle mixing. The bound protein on the matrix was rinsed with wash buffer (0.2% Triton X-100, 50 mM sodium phosphate, 300 mM NaCl, 35 mM imidazole) and a second wash buffer (0.1% Triton X-100, 50 mM sodium phosphate, 300 mM NaCl) was used to remove unspecific binding components. MscL was eluted from the nickel NTA column using an elution buffer (235 mM histidine, 0.1% Triton X-100, 300 mM NaCl, 50 mM sodium phosphate pH 8).
Reconstitution for conventional patch clamp

The MscL protein was reconstitution into artificial liposomes as explained before (19). Asolectin (soy extract 20%, Avanti polar lipids) was dissolved in lipid buffer (10 mM sodium phosphate and 150 mM NaCl, pH 8) at 20 mg/ml. The dissolved lipid was subsequently subjected to five freeze–thaw cycles in liquid nitrogen and 50°C water bath, respectively. Then the lipid vesicles were extruded through a 400 nm polycarbonate filter eleven times. A portion of the extruded lipid (250 μl) was mixed with 20 μl of 10% Triton X-100 detergent and incubated at 50°C for five minutes. Next, purified MscL protein (0.2-0.25 mg/ml) was added to the mixture of lipid (preformed liposomes) and detergent (1:50 protein to lipid weight ratio) and incubated for 30 min at 50°C. This was followed by addition of 200 mg of Biobeads™. The tubes were incubated overnight at 4°C with gentle mixing. The proteoliposomes were separated from the Biobeads™ and diluted to 0.8 mg/ml (lipid) with 2 mM MOPS buffer. The sample was spotted on two indium tin oxide (ITO) plates and dried in a desiccator for 2 hours under vacuum. Giant unilamellar vesicles (GUVs) were produced by rehydrating the lipid film using 250mM sucrose on Nanion’s Vesicle Prep Pro as explained before (11).

Reconstitution for automated planar patch clamp

GUV’s were prepared by electroformation using the Vesicle Prep Pro (Nanion Technologies) from DPhPC lipids as follows. 20 μl of lipid solution (10 mM DPhPC with 1mM cholesterol in trichloromethane) were spread out on an ITO slide. After evaporation of solvent, 270 μl of 1 M sorbitol were added and, electroformation was performed by alternating the voltage between x and y V at a frequency of 5 Hz for a period 2 hours. The obtained GUV’s were diluted with 200 μl of 1 M sorbitol. For reconstitution, 0.5 μl of detergent-solubilized purified protein were added to 200 μl of GUV containing solution, vortexed for two seconds and incubated at room temperature for 30 minutes. Subsequently the solution was diluted by 200 μl of sorbitol and incubated for two hours at 4°C. To remove the detergent that came with the pure protein, 10 mg of Biobeads™ were added and the sample was incubated over night at 4°C. After removing the Biobeads™ the GUV’s were stable for several days.

Generation of giant spheroplasts

Giant spheroplasts were prepared from an mscL-knock out E.coli strain PB104 based on Blount et al. protocol (17). An overnight culture using a single colony was prepared in 10 ml LB medium in the presence of ampicillin (100 μg/ml) and chloramphenicol (10 μg/ml). The following day, a fresh inoculum was prepared by making 1 to 50 dilution of the cells in LB medium supplemented with ampicillin (100 μg/ml) and chloramphenicol (10 μg/ml) antibiotics. After reaching an A600 of about 0.4, a 1:10 dilution was made into fresh LB medium with cephalexin (60 μg/ml). The cells were grown for 1 hour and expression was initiated by addition of arabinose (0.1% v/v). The cells were harvested by centrifugation at 4,000 rpm at 4°C for 10 minutes. The cell wall was digested by adding the following reagents sequentially: 120 μl of 1 M Tris-HCl pH 7.8, 120 μl of Lysozyme (5 mg/ml), 30 μl of DNAse (5 mg/ml) and 120 μl of 0.125 M EDTA pH 8. The digestion was allowed to proceed for 1.5 to 3 minutes and stopped by adding a stop solution (20 mM MgCl2, 10 mM Tris-HCL pH 7.8, 0.7 M sucrose). The solution was layered over a 13x100 mm glass tube containing 7 ml of 20 mM MgCl2, 10 mM Tris-HCL pH 7.8 plus 0.7 M sucrose. The spheroplasts were harvested by a 1 minute centrifugation at 4°C at 1500 rpm on a swing-out rotor. The spheroplasts were collected from the bottom 0.3 ml sucrose, aliquoted, and stored at -20°C.

Pipette pulling

Calibrated 100 μL pipettes (Drummond Scientific) with a 1 μm tip diameter were pulled using Sutter Instrument P-1000.

Conventional patch clamp of GUV’s

Single channel currents from GUVs were recorded following the standard patch clamp recording techniques (4). In brief, calibrated 100 μL pipettes (Drummond Scientific) with a 1 μm tip diameter were pulled using Sutter Instrument
P-1000. The pipette tip was filled with the same buffer as the bath (200 mM KCl, 90 mM MgCl₂, 10 mM CaCl₂ and 5 mM HEPES-KOH, pH 7). 2-5 μl of GUVs were added to a 160 μl of bath solution and gentle suction (up to 10 mmHg) applied to catch GUVs and also to make a gigaohm seal. When a gigaohmic seal was achieved, the pipette with the patch was briefly exposed to air and an inside out patch configuration was obtained. All recordings were performed under the same conditions (-20 mV, gain 10, sampling rate of 30 μs). The data were amplified and filtered at 10 kHz using an Axopatch 1D amplifier, sampled at 33 kHz in a Digidata 1322A digitizer, and analyzed with pCLAMP10 software (Molecular Devices).

**Automated planar patch clamp of GUV’s**

Experiments were done on the Port-a-Patch (Nanion Technologies). Buffer: 350 mM KCl, 10 mM HEPES-KOH, pH 7. NPC-1 patch clamp chips, 3-5 M Ohm with 5 μl of buffer on the internal and external side were used. After adjustment of voltage offset, 5 μl of GUV containing solutions were added to the chip. Bilayers with resistance of several Giga Ohm were formed due to fast application of 20 mBar (15 mmHg) negative pressure. 40 μl of buffer were added to the external side, holding potential was set to 20 mV and pressure was applied in stepwise manner (step width 10 mBar) with the suction control pro (Nanion Technologies) to evoke MSC currents.

**Conventional patch clamp of spheroplasts**

Spheroplasts were patched as GUV’s except that in order to form the Giga Ohm seal suction in the range of 10-15 mmHg was applied to the patch pipette tip. After seal formation between the spheroplast membrane and the pipette tip, and inside-out patch configuration was obtained, we first applied 45-55 mmHg negative pressure to activate MscS channels. We kept the tension constant and waited until MscS was desensitized. Afterwards, we applied more tension in a stepwise fashion in order to activate MscL channels.

**Automated planar patch clamp of spheroplasts**

Experiments were done on the Port-a-Patch (Nanion Technologies). Buffers: Internal: 300 mM KCl, 60 mM KF, 10 mM HEPES-KOH, pH 7; external: 350 mM KCl, 10 mM CaCl₂, 5 mM HEPES-KOH, pH 7; Seal buffer: 300 mM KCl, 40 mM CaCl₂, 10 mM HEPES-KOH, pH 7; Spheroplasts were diluted 1:2 with external buffer immediately before starting the experiment. NPC-1 patch clamp chips with resistance of 10-15 M Ohm were filled with internal and external buffers (5 μl internal, 10 μl external). 5 μl of spheroplast solutions were added to the external side, negative pressure was applied and increased till an increase of resistance indicated the attaching of an individual spheroplast to the chip. Giga Ohm seal was achieved due to release of pressure, application of negative voltage (up to -130 mV) and addition of 50 μl of sealing solution. After seal was formed, the external solution was completely exchanged to normal external buffer in three wash steps (removal and addition of 50 μl buffer). The holding potential was set to -20 mV, and increasing negative pressure applied to evoke MSC currents.

**Data analysis**

All analyses were performed using pClamp suite software (version 10.2; Axon Instruments, Foster City, CA). Port-a-Patch raw data were exported to Clampfit. Open probability was measured as a function of negative pressure and described by a Boltzmann distribution. Individual values were determined by integration of the current amplitude and normalization to the maximum activation. Dwell times were specified by means of single channel event detection. Time constants were calculated by fitting a logarithmic exponential probability function.
RESULTS AND DISCUSSION

The conventional patch clamp system comprises of a number of parts as shown in Figure 1A. The major parts being the amplifier, digitizer, and computer for detection and recording of the signal data; the inverted microscope with modified stage to hold the bath and patch pipette holder and the micromanipulator for aiding in visualizing and capturing of spheroplasts or GUVs. The conventional patch system requires the Faraday cage and the anti-vibrational table too (Figure 1A). Some of the major parts depicted in Figure 1A and 1B, which, compared to the conventional patch clamp system, have been markedly modified into a miniaturized unit in the automated patch clamp device (Figure 2A). In the Port-a-patch single channel automated patch clamp system (Figure 2A), the classical patch pipette (Figure 1A-2 and 1B-2) is replaced with a flat horizontal borosilicate glass surface with a defined size micro structured hole (Figure 2B) that separates two individually addressable compartments, i.e. cis and trans compartments. The cis compartment, which represents the external bath, is fully accessible, whereas the trans compartment with internal solution is hermetically sealed, so that pressure can be applied (Figure 2C). The membrane of interest is brought into the hole by applied suction through a pressure pump, where it forms a gigaohm seal. The diameter of the pore varies from less than one to several micrometers, depending on the intended application. The important element of the system that makes it suitable for patching mechanosensitive ion channels is the presence of the controlled pressure pump that can apply negative or positive pressure in the range of -300 mBar to 300 mBar (225 mmHg), manually or by means of computer generated pressure protocols, with the minimum step size of 1 mBar (0.75 mmHg). Once a gigaohm seal is formed between the membrane and the pore, as in conventional patch clamp, the suction is released with no loss of the seal resistance. Subsequently, mechanosensitive channels are activated by applying the desired pressure protocols.

We first worked with reconstituted wild type MscL in lipid bilayers generated from GUV’s. For conventional patch clamp, we generated giant asolectin proteoliposomes by electroformation of reconstituted large unilamellar liposomes, as explained before (11). We performed patch clamp using the same buffer both in the bath and the pipette (Figure 1A-2). The buffer was composed of 200 mM KCl, 90 mM MgCl₂, 10 mM CaCl₂, 5 mM HEPES-KOH, pH 7. In a typical patch, we obtained 2-5 Giga Ohm resistance and could activate MscL channels by applying suction in the range of 60 to 100 mmHg (Figure 3A). The single channel conductance was 3.2 nS in this buffer. The probabilities of open channel within a patch relative to the amount of negative pressure were fitted with a Boltzmann distribution according to Eq. 1 (12):

\[ \frac{P_o}{P_c} = \exp (\alpha (p - P_{1/2})) \]

where,

- \( P_o \) is the probability of an open channel,
- \( P_c \) is probability of closed channel (1-\( P_o \)),
- \( p \) is the applied negative pressure,
- \( P_{1/2} \) is the negative pressure applied to the pipette at which MscL channels open 50% of the time,
- \( \alpha \) is the slope of the plot \( \ln(P_o/P_c) \) versus \( (p-P_{1/2}) \).

Conventional Patch Clamp

In conventional patch clamp the \( P_{1/2} \) of MscL was 104.8 ± 0.3 mmHg ± (±SE, N=3) (Figure 3B). The sensitivity of the channels to the applied negative pressure \( (1/\alpha) \) was 5.2 ± 1.29 mmHg (± SD, N=3) per \( e \)-fold change in the open probability, in agreement with published data (~4.5 ± 2.00 mmHg, N=5) (9, 13). The distribution of the open dwell times could be fitted with a three components model and revealed time constants (Figure 3C).
Figure 1. Conventional Patch clamp system. (A) A picture of the patch clamp setup showing the main components: (1) inverted microscope, (2) bath and patch pipette, (3) micromanipulator, (4) pressure monitor, (5) amplifier, (6) digitizer, (7) computer, (8) vibration isolation table, and (9) Faraday cage. One electrode is placed inside the pipette, which is filled with buffer, and the second one is located in the bath with the same buffer. Once a patch with gigaohm seal is made, single channel currents are amplified in the voltage-clamp mode and converted to a digital readout by the digitizer as depicted on the computer monitor. (B) Zoom in on A2 to show bath (1) and micropipette (2) which correspond to the NPC1 chip in the automated planar patch clamp (Fig 1A).

In case of automated planar patch clamp, we used 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) as the lipid and generated GUV’s with no MscL channel from DPhPC using electroformation. We reconstituted the protein by adding detergent-solubilized MscL to a suspension of preformed liposomes and removing the detergent by Biobeads™; GUVs were subsequently formed by electroformation. We adapted the buffer condition for the automated patch to 350 mM KCl, 10 mM HEPES-KOH, pH7 and we eliminated magnesium and calcium ions. Due to the horizontal placement of the pore in the automated patch setup, the presence of high concentrations of these divalent cations, as it is in the conventional patch buffer, would facilitate the fusion of other GUVs into an already existing membrane on the pore. We used patch clamp chips with an aperture of 1 μm. Under these conditions, we could obtain seals with typically several tens of Giga Ohm resistance.
Automated planar Patch Clamp

Figure 2. Functionality of the automated planar patch clamp “Port-a-Patch”. (A) Port-a-Patch connected to suction control unit (Suction Control Pro). (B) Microscopic picture of NPC1 patch clamp chip. (C) Diagram of technical setup. Input parameters voltage and pressure are constantly reported back to a computer (PC) via the HEKA patch clamp amplifier and in that way precisely controlled.

When we applied tension from 50 to 90 mmHg in a stepwise manner with 10 mmHg step size, using pressure protocols generated with Patch Master (HEKA), we could activate MscL channels (Figure 3D). We defined the $P_{1/2}$ as $77.5 \pm 2.7$ mmHg (±SE, N=3) (Figure 3E), which was lower than that obtained from the conventional patch clamp, i.e. 104.4 mmHg. Since MscL senses the tension (14) $P_{1/2}$ is a function of not only the applied negative pressure but also the patch radius, and thus our results suggest that the patch radius in automated patch clamp is larger than in the conventional system. However, the sensitivity of the channels to the applied negative pressure, which is expected to be independent of the half activation pressure (15), was similar to that obtained from the conventional patch clamp, i.e. $4.78 \pm 1.34$ mmHg (±SD, N=3). Furthermore, the analysis of open channel dwell times revealed similar time constants to that obtained from the conventional system (Figure 3F). The observed conductance on the automated planar system was 3.6 nS.
After reconstituting MscL in giant liposomes with the automated patch clamp, we set out to patch clamp a more challenging sample, i.e. bacterial cells. Because of the small size and the presence of a double membrane and cell wall in \textit{E. coli} cells, patching is possible only in giant spheroplasts obtained from them. Giant spheroplasts are 5-10 \( \mu \)m in diameter, consist of a cytoplasmic membrane and disrupted cell wall (7). Conventional patch clamp of giant \textit{E. coli} spheroplasts requires a strong suction for tens of seconds to generate a Giga Ohm seal (Table 1). Once the seal is formed, the suction is released without losing the seal (7). We generated spheroplasts from \textit{mscL}-null \textit{E. coli} cells. These cells do not have MscL but posses other MSC, for example mechanosensitive channel of small conductance (MscS). MscS is more sensitive to tension than MscL therefore its activity in the patch clamp appears at much lower pressures. It has been shown that the ratio of negative pressure threshold of activating MscL to that of MscS is about 1.5 and is independent of the patch geometry (16). In our work, we used MscS as an internal control for comparing MscL activation threshold in two different patch clamp setups. Spheroplasts were generated as explained before (17), and they were used in both conventional and automated planar patch clamp recordings. For conventional patch clamp, 2-5 \( \mu \)l of spheroplasts were added to 160 \( \mu \)l of a bath solution (200 mM KCl, 90 mM MgCl\(_2\), 10 mM CaCl\(_2\), 5 mM HEPES-KOH, pH 7). In order to make the seal between the tip of the pipette and the spheroplast membrane, a gentle
suction in the range of 15-20 mmHg was applied to the pipette tip. Recordings were done in excised inside-out patches with seal resistance of 2-5 Giga Ohm. At the beginning low negative pressure (45-55 mmHg) was applied to activate MscS channels. MscS has a conductance of approximately 1 nS. After inactivation of MscS channels, the negative pressure was increased to activate MscL channels (Figure 4A). In accordance with the literature (20) the ratio of pressures necessary to activate MscL to MscS was 1.5 ± 0.2 (± SD, N=10). The conductance of MscL channels was 3.5 nS. The midpoint pressure for activation was 97.3 ± 0.4 mmHg (± SE, N= 3), whereas the sensitivity to negative pressure 1/\alpha was 6.4 ± 2.7 mmHg (± SD, N= 3) (Figure 4B). The channel kinetics are given in Figure 4C.

Figure 4. Conventional and automated planar patch clamp of spheroplasts. (A) and (D) Left panel: channel activity of MscS and MscL at -20 mV in conventional and at 20mV in automated patch clamp, respectively. Both channel activities are indicated as upwards deflections. Right panel: enlarged view of MscL channel activity. (B) and (E) Open probability of MscL as a function of applied negative pressure in conventional and automated patch, respectively. (C) and (F) Single channel dwell time analysis of MscL in conventional and automated patch, respectively. Channel open dwell times were fitted with three time constants and values are given as the mean ± SE.
On the automated planar patch system we diluted the spheroplast preparation 1:2 in the external buffer (350 mM KCl, 10 mM CaCl₂, 10 mM HEPES-KOH, pH 7), and added 5 μl of it onto 10 μl external buffer already on the chip, while a gentle pressure in the range of 10 to 50 mmHg was applied to catch a spheroplast. Once a spheroplast was caught, as evidenced by increasing resistance, the suction was relieved and the sealing process continued without pressure. When a seal starts to form, we added 50 μl of a sealing solution to the *cis* compartment similar to the external buffer but with increased calcium concentration (40 mM), which helps to form a high seal resistance. The internal buffer was 300 mM KCl, 60 mM KF and 10 mM HEPES-KOH, pH 7. KF has long been known to enhance the seal resistance (18). Once the Giga Ohm seal was formed, the buffer on the *cis* compartment was replaced with fresh external buffer. The use of different buffers that are the external and internal buffer together with the sealant is essential to formation of gigaohm seals in the automated planar patch system unlike the conventional patch system where one buffer suffices (Table 1). For spheroplasts, we used chips of a smaller pore diameter (< 0.5 μm). Channel recordings were done in the “cell attached” mode with a resistance of at least one Giga Ohm. In the same way as described for conventional patch clamp, first MscS channels opened at 35 mmHg negative pressure (Figure 4D), after they were inactivated, more negative pressure was applied to activate MscL. The ratio of pressures was calculated as 1.8 ± 0.4 (±SD, N=14), which is comparable to that obtained from the conventional patch clamp recordings. The conductance of MscL was 3.1 nS. The P½ of 106 ± 6.6 (±SE, N=5), the sensitivity to applied tension of 4.71 +/- 2.34 (±SD, N=5) and channel kinetics were similar to that obtained by the conventional system (Figure 4E and F). It is interesting to note the close agreement of the results obtained using the two patch clamp systems, even though there is some obvious design difference and system requirements (Table 1). Some of the differences outlined in Table 1 may have cost implication in the overall running of a particular patch clamp system, however for most researchers how fast one can obtain the desired result will be the overriding consideration.
Table 1. Summary of the difference between conventional and automated planar patch clamp.

<table>
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<tr>
<th>Patch Clamp type</th>
<th>Consumables</th>
<th>Accessibility of the membrane</th>
<th>Suction</th>
<th>Visualization (when sealing)</th>
<th>Accessory accessories</th>
<th>Operator's input</th>
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<tbody>
<tr>
<td>Conventional Patch Clamp</td>
<td>Glass pipettes</td>
<td>Directly from bath side or with back filling From inside patch pipette</td>
<td>Manual and automated</td>
<td>Microscope present</td>
<td>-Pipette puller -Vibration isolation table</td>
<td>necessary</td>
</tr>
<tr>
<td>Automated Planar Patch Clamp</td>
<td>NPC 1 chips</td>
<td>Directly from either side of the membrane</td>
<td>Automated</td>
<td>optional</td>
<td>Perfusion system for buffer exchange</td>
<td>limited</td>
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Remark

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<tr>
<th>Disposable in both conventional and automated patch clamp</th>
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<tr>
<td>Automate d system needs sealant (seal enhancer)</td>
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<tr>
<td>Suction precision in the conventional patch clamp is low</td>
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<tr>
<td>False gigaohm seal is easily identifiable by vision in the conventional patch clamp</td>
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<tr>
<td>Automat ed Planar patch system is less depende nt on the operator’s skill</td>
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In summary, we successfully patch clamped mechanosensitive ion channels on a small, automated planar patch clamp system and could reproduce the single channel parameters for MscL. The system requires minimal user input and is suitable for patching not only synthetic lipids but also bacterial giant spheroplasts. Developing a simpler and more efficient alternative to conventional patch clamp to investigate mechanosensitive channels will enable not only fundamental studies but also will facilitate high throughput screening of drugs for MSCs.
References