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Experimental and simulation study of the effect of pipette roughness on giga-seal formation in patch clamping

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ABSTRACT

The effect of pipette tip roughness on giga-seal formation of patch clamp recording has been studied through FEA simulation and patch clamp experiments. FEA simulation results show that the membrane cannot fill up all of the peaks and valleys of a rough pipette tip. As a result in three dimensions the seal between inside and outside is compromised by channels in the order of several nanometres. These channels increase the leakage current between two electrodes, increase the noise and decrease the seal resistance. In contrast focused-ion-beam polished pipettes have very flat tips. Single ion channel currents recorded by FIB polished pipettes show significantly smaller leakage current and noise than the currents recorded by conventional pipettes. Results of FEA simulation are consistent with the results of patch clamp experiments.

1. Introduction

Introduced by Neher and Sakmman, patch clamp technique has been widely used for studying cellular ion channels [1]. Nowadays, it has been proven that many different diseases can be caused by the mis-function of ion channels [2]. In patch clamping a patch of membrane is isolated from external solution to record the currents flowing into the patch. To achieve this, small glass capillaries are heated and pulled to fabricate glass micropipettes with a tip diameter of 1–2 μm. The pipettes are then backfilled with a conductive solution and pressed against the surface of a cell. To improve the sealing condition a gentle suction is applied to the backend of the pipette. As it is shown in Fig. 1 there are two electrodes in patch clamp set-up: a recording electrode inside the pipette and a reference electrode in the bath solution. A high resistance seal between the glass and the patch of membrane reduces the leakage current between the two electrodes and completes the electrical isolation of the membrane patch. It also reduces the current noise of the recording, permitting good time resolution of single-channel currents which are in the order of 1 pA [3]. Since the electrical resistance of the seal is in the order of giga-ohms, it is called giga-seal. In this paper a study in the effect of the pipette tip roughness on giga-seal formation is presented. The study was conducted through repeated patch clamp experiments and finite element modelling and analysis.

2. Patch clamp manipulators

Patch clamping involves the placement of a glass micropipette onto a cell to form a tight seal. The core function of a micromanipulator in patch clamping set-up is to place the micropipette tip onto the cell surface in a controlled way [3]. The manipulator used in the experiments is MP-225 from Sutter Instrument, which has a minimal resolution of 62.5 nm for fine movements. As a result the pipette approaches the cell in a step of 62.5 nm. This implies that when a contact is made, the pipette tip is just on the cell surface or presses the cell within tens of nanometres. If the pipette continues its movement, it may penetrate or rupture the cell membrane. Fig. 2 shows the right moment of applying suction. In practice the relative position of tip to membrane is estimated by monitoring the change of electrical resistance between the two electrodes. As the pipette comes closer to the membrane the resistance increases; usually an increase of about 1 MΩ indicates that the tip has touched the membrane [3]. It is not recommended to reach a resistance 1.5 times higher than the pipette resistance as it stresses the membrane and contaminates the tip which is fatal for giga-seal formation. In the finite element modelling, once in contact, the pipette is lowered by 100 nm and the suction is applied then.

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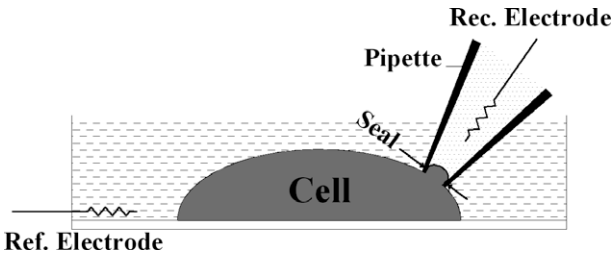

Fig. 1. The schematic of the patch clamp method.

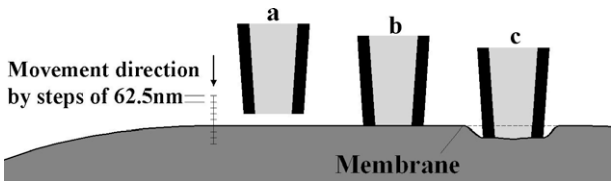

Fig. 2. The pipette approaches the cell by steps of as long as manipulator resolution. (a) The pipette is far from the membrane, (b) the pipette is on the membrane; this is the best position to apply suction and (c) the pipette presses the membrane by few tens of nanometres; this is the usually occurred case in patch clamping.

3. FEA modelling

The FEA modelling is proposed to find out how a cell deforms under the rough tip of a pipette. Fig. 3 shows an SEM image of a glass micropipette and its Digital Elevation Model (DEM) image created by Mex (SimpleWare), a software package specialized in processing SEM images [4]. The surface roughness of the pipette tip (S_a) is 27 nm measured by 3D reconstruction method [5].

Fig. 3 also shows four different profiles (numbered 1–4) of the tip surface along its thickness. The specifications of the profiles are given in Table 1. In order for the modelled pipette to have the real tip profile of the glass micropipette, coordinates of each profile are extracted and a B-spline is drawn through these points in CATIA. The splines are then transferred to ABAQUS for finite element simulation. The inner and outer diameters of the pipette tip are 0.7 μm and 1 μm, respectively. The micropipette is modelled as a rigid solid.

The cell is modelled as a continuous, homogeneous, isotropic elastic solid attached to the substrate [6,7]. The cell consists of three parts: the cytoplasm, actin cortex and membrane. The cytoplasm is modelled as a quarter plane meshed with 4-node bilinear axisymmetric quadrilateral elements. The cortex and plasma membrane are modelled as thin elastic shells. The area of interest is the region below the tip and regions far from the pipette will not be affected. Consequently, only the cellular region within a 2 μm of the axis of the pipette is modelled. Material properties and dimensions used for finite element modelling are summarized in Table 2. Simulation was carried out with commercial finite element analysis software, ABAQUS, using 2D axial symmetry mode and explicit dynamic formulation code [8]. As shown in Fig. 4 a the cell is not allowed to move on the downside but it is only allowed to move in the y direction along the axis of symmetry. In the finite element simulation the pipette is lowered for 100 nm and then the suction is applied to the inside of the pipette.

The result of the finite element modelling is shown in fig. 4. Although the cytoplasm is soft, the stiffer membrane does not allow the cell to cover all of the cavities of the tip. The highest peaks of the profile push down the membrane. Therefore, the distance between two peaks will not be filled with the patched membrane. This is important because, if the membrane fills up all of the room between peaks and valleys then the contact area between the membrane and the glass will increase. This in turn can result in a better seal. But the result of FEA modelling shows that the membrane cannot go into the valleys. Therefore the room which is left over, acts like channels connecting inside and outside of the pipette together. Theses channels are filled with the conductive media, making it easier for ions to escape, therefore increasing the leakage current and compromising the seal. As it can be understood from Table 1 the average heights of these channels are about 8–23 nm.

4. Experiments

The uneven surface of the pipette tip was corrected by cutting the top of the pipette across using FEI dual beam focused ion beam system. In the FIB milling process, the tips of pipettes were cut using Ga^+ ions with 50 pA current for 100 s and dwell time of 1 μs. The conditions of a pipette tip before and after milling are shown in Fig. 5a and b. The image in Fig. 5b has a resolution of 4.5 nm. No feature could be identified on the milled surface for producing roughness parameters at this magnification. Therefore, the average surface roughness (S_a) of the milled pipette tip should be less than 4.5 nm.

Patch clamping experiments were carried out on HUVECs (Human Umbilical Vein Endothelial Cell) using both conventional and the FIB polished micropipettes for achieving giga-ohm seals. HUVECs were cultured in EBM medium (Lonza Co., CC-3121) on cover slips two to three days before the experiments and incubation was done at 37 °C. HUVECs are well known for their extremely flat shape making them one of the most difficult cell types for patch clamping. Experimental equipment setup consisted of Axon 1D amplifier, Flaming/Brown micropipette puller (P-97, Sutter Instrument) and glass micropipettes (BF150–86-10, Sutter Instrument). The average opening of the pipette tips was about 1.4 μm in diameter. The backfilling solution was composed of 40 mM KCl, 96 mM K-gluconate, 4 mM K_2ATP, 2 mM GTP, 10 mM HEPE and at 7.2 in pH, and the bath solution was composed of 110 mM NaCl,, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPEs, 5 mM HEPE–Na, and at 7.2 in pH.

Pulses (10 mV) were constantly applied on the recording electrode from the time that pipette tip was just immersed in the bath solution till it touched the cell membrane. A negative pressure was

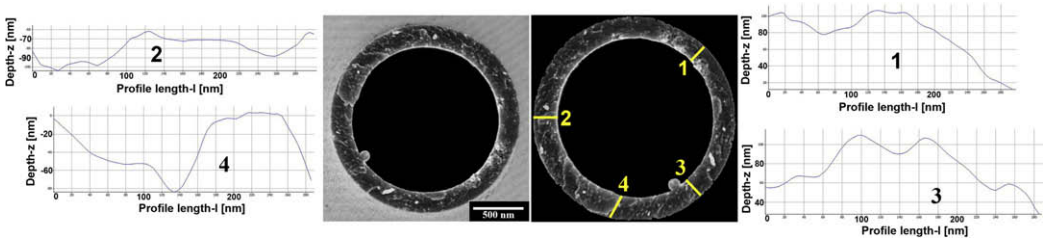

Fig. 3. SEM image of glass micropipette (right) and DEM image of the tip (left); four profiles of the tip thickness are also given.