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Characterization of a MEMS BioChip for planar patch-clamp recording

Santosh Pandey *, Rajiv Mehrotra, Sherri Wykosky, Marvin H. White *

Department of Electrical Engineering, Sherman Fairchild Center, Lehigh University, 16A Memorial Drive East, Bethlehem, PA 18015, USA

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Abstract

We describe a planar MEMS silicon structure to record ion channel currents in biological cells. The conventional method of performing an electrophysiological experiment, 'patch-clamping', employs a glass micropipette. The micropipette tip is a source of thermal noise because of its inherent, tapered, conical structure, giving rise to a large pipette resistance. This pipette resistance, when coupled with the biological cell capacitance, limits the available bandwidth of single ion channel recording. In this work, we propose a current transport model to characterize the series resistance and capacitance of a planar pipette fabricated on a silicon BioChip. Our model provides a deeper insight into how currents injected into a micropore are quantitatively partitioned into the individual ion transports, and goes beyond just describing the solute and solvent kinetics inside pores of microscale dimensions. The device topology and fabrication sequence of the planar patch-clamp setup are also discussed. The theoretical predictions by the model are in close agreement with the experimental results.

Keywords: Patch-clamping; Microelectromechanical systems (MEMS); Glass micropipette; Current transport model

1. Introduction

Miniaturized bio-chemical measurement systems will have a large impact on the way medical diagnostics will be performed in the future, particularly in clinical diagnostics and pharmacology where the use of living cells for fast specific and non-specific chemical sensing is an area of increasing importance [1,2]. A cell or cell layer is a complex system with appropriate response to a variety of external physical and chemical excitations. Experiments on such living biological systems can lead to the study of diffusion and transport of biological or pharmacological molecules through the cell or cell layer. The ion-transport mechanisms in living cells are governed by the opening and closing of microvalves in the cell membrane called 'ion channels'. These ion channels use the energy stored in chemical concentration gradients to produce small electrical signals during the movement of charged ions.

The patch-clamp technique is a method for studying the ion channel behavior where a small 'patch' of the cell membrane is isolated for localized electrical measurement by placing a glass micropipette onto the surface of a voltage-clamped cell [1,2]. The patch-clamp setup and the electrical model of the cell are shown in Fig. 1. A biological cell has ion channels, each type of ion channel being represented by a conductance G_K and a resting potential V_K. The cell membrane contributes a capacitance C_M and the seal formed between the patch and the glass pipette is represented by a conductance G_S. The glass pipette has a resistance R_P and a capacitance C_P associated with it. The electrical parameters of the cell

* Corresponding authors. Tel.: +610-758-4518 (S. Pandey); +610-758-4421 (M.H. White).
E-mail addresses: skp3@lehigh.edu (S. Pandey), mhw0@lehigh.edu (M.H. White).

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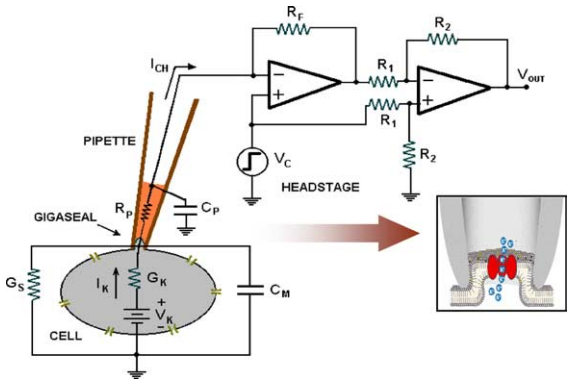

Fig. 1. This figure shows the conventional method of doing patch-clamp experiments. A glass pipette, which has the testing medium, is placed on the cell and a gentle suction is applied to form a gigaohm seal between the pipette tip and the cell membrane. The electrical model of the cell and the pipette is also shown in the figure. A command voltage V_C is applied to the cell through the feedback action of the input operational amplifier. The measured signal goes to a transimpedance amplifier, where it is amplified and fed to an A/D converter. The inset shows a magnified image of the seal formation, with ions being exchanged through an ion channel in the cell membrane.

are not constant but may vary during the course of any patch-clamping experiment. Typical values for a 10 μm cell are: $G_K = 10^{-9}\ \Omega^{-1}$, $V_K = 56$ mV, $G_S = 1 - 2 \times 10^{-11}\ \Omega^{-1}$, $C_M = 10 - 20$ pF, $R_P = 10^7\ \Omega$, $C_P = 0.01$ pF.

In this work, we explore the transport of ions through a micropore as a precursor to the fabrication of an integrated planar patch-clamp measurement system on a microelectronic BioChip. Such a BioChip has the advantages of system integration (wells and electronics), cost-effectiveness and high-throughput-screening (HTS) [3]. Various groups have attempted to measure ionic currents through biological cells on platforms, similar to the one described in this work.

In planar patch-clamping, the introduction of a planar pipette on a silicon chip is expected to reduce the intrinsic resistance, thereby opening the possibility of recording faster switching ion channel currents. It is to be mentioned that the micropore serves as a fluid conduit between two chambers and could be pictured as a planar pipette (Fig. 2). Furthermore, since the BioChip capacitance is in parallel to the capacitance due to the cell membrane, it is crucial to estimate the chip capacitance, and limit it to a value lower than that of the cell membrane. To our knowledge, there is no available analytical model to precisely estimate the series resistance of a planar pipette or the net capacitance of such a BioChip.

Here, we develop a comprehensive, yet simplistic model to characterize the electrolyte-filled micropores with respect to their geometrical, electrochemical and electrical properties. Basically, the current-carrying properties through the micropore are governed by the laws of electrodiffusion and electro-osmosis operating on the ionic components of the electrolyte solutions. However, our model provides a deeper insight into how currents injected into the micropore are quantitatively partitioned into the individual ion transports, and goes beyond just describing the solute and solvent kinetics inside pores of microscale dimensions. Furthermore, we present a simplistic capacitance model to account for the different factors contributing to the net capacitance of the BioChip.

2. Device fabrication

The fabrication process of the BioChip is a two-mask process and was carried out at the Microfabrication Laboratory, Lehigh University. We start with a 375 μm

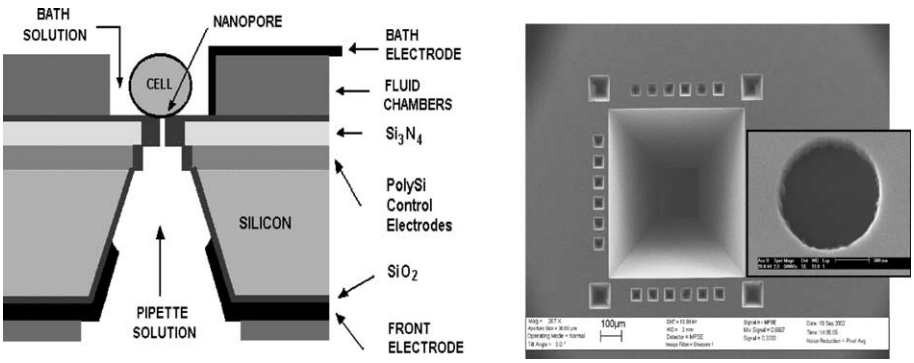

Fig. 2. The figure on the left shows the topology of the device fabricated for planar patch-clamping. The figure on the right is a SEM picture of the suspended nitride membrane in an anisotropically-etched, silicon well. The inset shows a nanopore drilled in the nitride membrane.

p-doped ⟨100⟩ double-sided polished silicon wafer. After RCA cleaning, a 2000 Å SiO_2 and a 1000 Å Si_3N_4 are deposited on the silicon wafers. The first photolithography step follows, where the backsides of the wafers are opened by etching the SiO_2/Si_3N_4 layers for the formation of the wells. The wafers are then immersed in a 24% KOH solution at 80 °C for 5 h, with occasional stirring to dislodge the etched silicon debris from the wafers. Subsequent anisotropic etching of silicon in aqueous KOH solution resulted in a V-shaped groove, where the upper Si_3N_4 membrane served as an etch-stop. After the formation of ultra-deep silicon wells with suspended Si_3N_4 membrane, a second photolithography step follows, where the frontside is exposed in alignment with the etched backside wells. Plasma etching of the frontside Si_3N_4 membrane creates micropores of varying diameters. After the entire microfabrication sequence, the BioChip as shown in Fig. 2, consists of a micropore (3–20 μm) in a nitride membrane (1000 Å thick) resting on an ultra-deep silicon well (375 μm deep). After dicing the wafers to appropriate chip sizes, both sides of the chips were bonded to 250 μm-thick poly-dimethylsiloxane (PDMS) gaskets with 4 mm^2 openings. Teflon structures are also made of suitable dimensions to serve as fluid chambers (<100 μl), with O-ring seals to prevent any spill of the electrolyte solutions. A LabView controlled HP4145a Semiconductor Parameter Analyzer provides the necessary voltage for the experimentation to the Ag/AgCl microelectrodes probes. Also, a LabView controlled HP4192A Impedance Analyzer is connected to the probes to measure the net capacitance and conductance of the BioChip.

3. Current transport model

In planar patch-clamp experiments, it is crucial to characterize the inherent series resistance and capacitance of the chip substrate. Low values of distributed RC noise are critical in obtaining highly accurate single-channel cell-attached recordings. Therefore, keeping the parasitic capacitance and series resistance of our substrates as low as possible is important for obtaining single-channel recordings. Noise from parasitic capacitances can easily exceed the current generated by a single-ion channel. In whole-cell recording, it is best for additional parasitic capacitance of the system to be less than the capacitance of a whole cell (10–100 pF), so that the cell membrane is the dominant noise factor.

For the above reasons, we have developed an analytical model to estimate the series resistance and the capacitance of a planar patch-clamp structure. To start, the net current of a particular ion (cation or anion) flowing through a micropore (in Fig. 3) is the sum of its drift and diffusion components [4,5] and is written as:

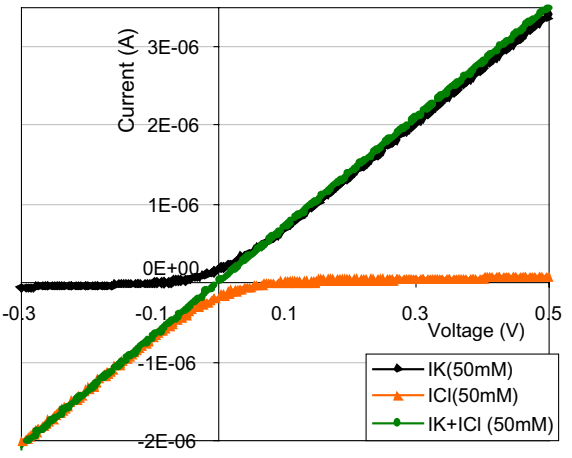

Fig. 3. Calculated individual K^+, Cl^- current components for KCl electrolyte and the summed current.

$$I = A\Im Dz[(\mathrm{d}c_i/\mathrm{d}x) + (z\Im c_i/\Re T)(\mathrm{d}\Phi/\mathrm{d}x)] \quad (1)$$

where I is the electro-diffusive ionic current, z is the ion valency, D is the ionic diffusion coefficient, $\Im$ is the Faraday's constant, $\Re$ is the gas constant, T is the temperature, A is the cross-sectional area of the micropore, c_i is the ionic concentration, and $-\mathrm{d}\Phi/\mathrm{d}x$ is the applied electric field. If we assume a linearly-varying potential, $\Phi = \Phi_0(1 - x/d)$ with Φ_0 being the applied potential, then the ion current flow through a micropore is:

$$I = (A\Im Dz\varsigma/d)[(c_2 - c_1 e^{-z\varsigma})/(1 - e^{-z\varsigma})] \quad (2)$$

where $\varsigma = \Im\phi_0/\Re T$, d is the region of potential variation, and c_1, c_2 are the ionic concentrations in the pipette and bath, respectively (Fig. 2). For the case of ionic conduction using KCl electrolyte, the individual ionic current components are:

$$I(\mathrm{K}) = (A\Im D\varsigma/d)[(c_2 - c_1 e^{-\varsigma})/(1 - e^{-\varsigma})]$$
$$I(\mathrm{Cl}) = (-A\Im D\varsigma/d)[(c_2 - c_1 e^{\varsigma})/(1 - e^{\varsigma})] \quad (3)$$

Fig. 3 plots the individual current components of KCl electrolyte and their summation employing Eq. (3). In order to estimate the total current through a micropore, we need to add an additional correction factor $(\sigma\Re T/\Im)\ln(c_2/c_1)$, which is the product of conductance σ and the Nernst potential [6].

$$I_{\mathrm{theoretical}} = I(\mathrm{K}) + I(\mathrm{Cl}) + (\sigma\Re T/\Im)\ln(c_2/c_1) \quad (4)$$

Fig. 4 shows the experimental current–voltage plots at various KCl concentrations through a micropore using tungsten probes for electrical stimulation. The non-linear nature of these plots suggests that tungsten probes are not suitable for such measurement. For our later experiments, we use Ag/AgCl probes as they are

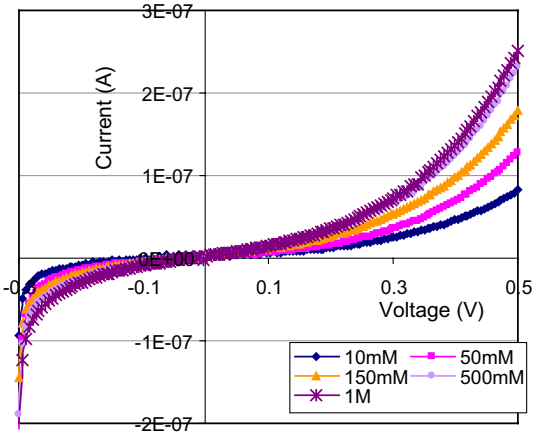

Fig. 4. Experimental, non-linear current–voltage curves obtained using tungsten probes. Only electrodes which are capable of ion-exchange, such as Ag/AgCl, are suitable for these experiments.

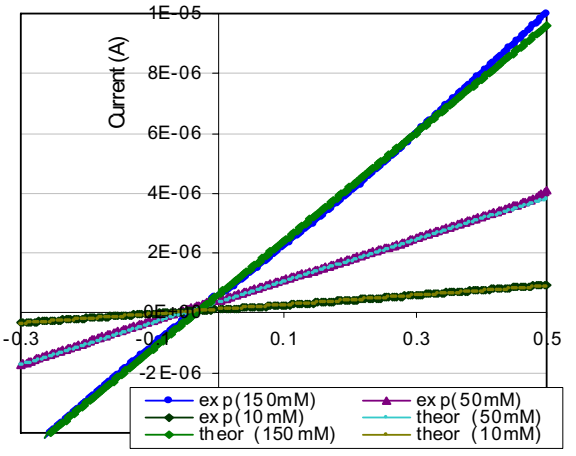

Fig. 6. Comparison of the theoretical total current with the experimental data at (10 mM, 500 mM), (50 mM, 500 mM), (150 mM, 500 mM) KCl.

known to be ion-exchange electrodes. In Fig. 5, the experimentally measured total current for (50 mM, 500 mM) KCl and (150 mM, 500 mM) KCl are compared with the theoretical current, with and without the correction factor. From the figure, it is observed that the theoretical current without the correction factor has no threshold voltage, even though its slope is in close agreement to the experimental plot. After adding a correction factor, as in Eq. (4), there is a good match between the theoretical current and the experimental data. This is because there is an inherent voltage of the order of 25–70 mV developed in the micropore even at zero applied voltage. This inherent voltage is needed for experimental fitting of the theoretical current is precisely as predicted by the correction factor in Eq. (4). Fig. 6 does the same comparison for three (10, 50 and 150 mM with 500 mM) KCl concentrations. There is good agreement between the experimental data and the theoretical model presented. The cross-sectional area, the thickness of the membrane, and the electrolyte concentrations are key factors in determining the series resistance of the chip (as shown in Fig. 7).

The net capacitance of the chip is a sum of the nitride-oxide layer capacitance of the wafer (C_1), and that of the nitride membrane (C_2). Using an oxide thickness of ~3000 Å and a nitride thickness of ~1000 Å, the effective oxide thickness is ~3520 Å. The net capacitance is: $C_{\text{total}} \cong 695$ pF, with $C_1 \cong 693$ pF and $C_2 \cong 1.7$ pF. Fig. 8 shows the experimental capacitance

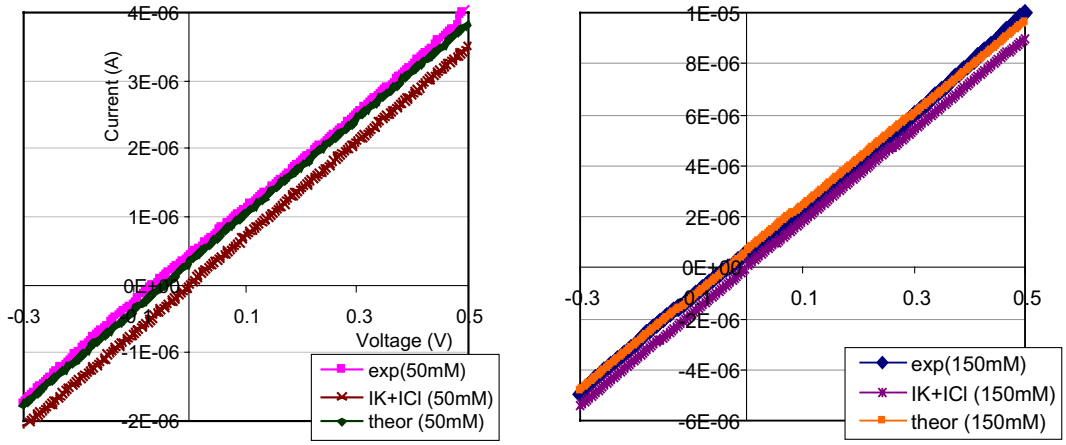

Fig. 5. Comparison of the theoretical total current (with and without correction factor) with the measured data for (50 mM, 500 mM) KCl and (150 mM, 500 mM) KCl.

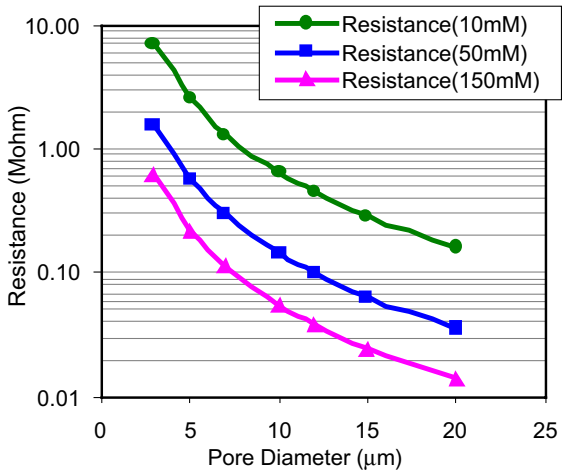

Fig. 7. Measured resistance of the micropores of varying sizes at 10, 50, and 150 mM KCl.

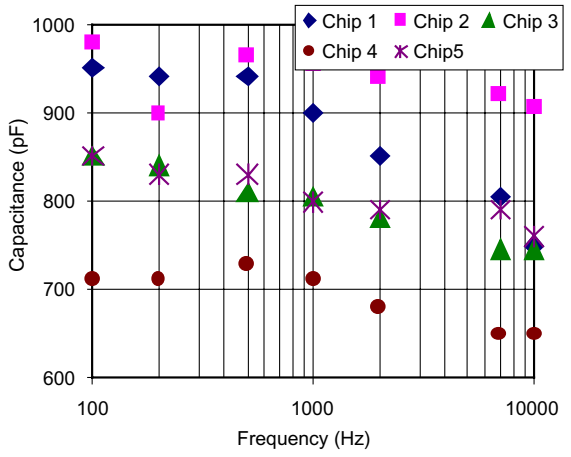

Fig. 8. Measured capacitance of the fabricated device at varying frequencies.

values of the fabricated chips. The measured capacitance is ~800 pF, which is reasonably close to the calculated value of 693 pF; however, this substrate capacitance is higher than a cell-membrane capacitance (~100 pF), which can be reduced by minimizing the fluid area in contact with the chip.

4. Conclusion and future work

In conclusion, we present theoretical models for the net series resistance and capacitance associated with the flow of ions through a micropore—an integral part of an integrated planar patch-clamp silicon device. The effective series resistance is around 1 MΩ for (150 mM, 500 mM) KCl and 2 μm diameter micropore, while the net capacitance is around 800 pF. These models reflect the interdependence of key physical parameters of the structure in determining the above chip resistance and capacitance. Our models agree well with the experimentally measured data.

Some of the challenges involved in performing a successful patch-clamp experiment on-chip are the automated positioning of cells on the orifices, the biocompatibility of the chip surface, the formation of a gigaohm seal between the cell membrane and the orifice, and the reduction of noise during the measurements. To this end, the fabricated chip also has various quadruple electrode structures, made of Ti/Pt, surrounding the micropore for automated trapping of a single cell at the desired site. Since the surface of the most native vesicles and cells bear electrical charge, properly directed electrical fields can provide precise electrophoretic positioning on the pore drilled in the membrane. By applying an a.c. voltage of appropriate amplitude and frequency to the electrodes, strongly inhomogeneous fields can be created around the micropore used for positioning, resulting in a focused movement of a single cell towards the point with lowest electric field, and its

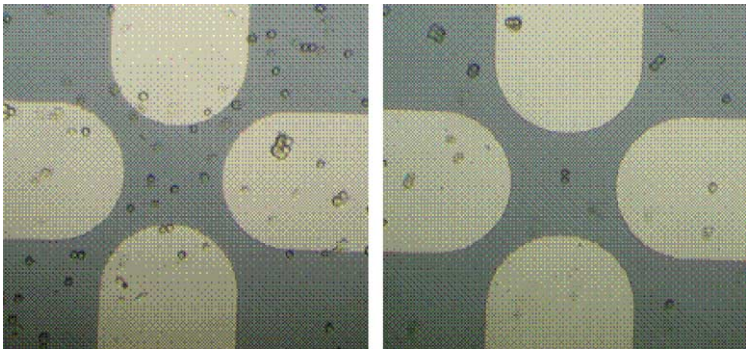

Fig. 9. Dielectrophoretic forces can be employed to trap single cells at a desired site on the chip.

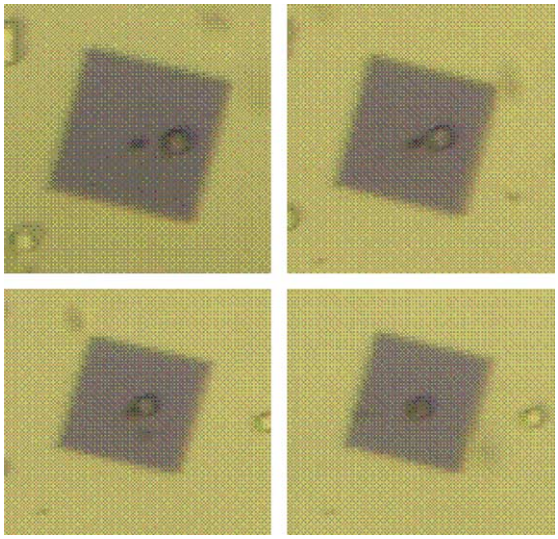

Fig. 10. A single cell can be attracted towards a micropore by applying a negative pressure from underneath the chip.

trapping thereafter (Fig. 9). The use of negative pressure from underneath the chip can also attract the cells to the micropore, provided it is localized around the micropore (Fig. 10). It was seen that adequate chemical modification of the chip surface by poly-D-lysine facilitated cell adhesion. Also, adding ions like phosphates, sulfates and calcium, and fetal-bovine-serum seemed to increase the conductivity and cell motility of the cell suspension. A combination of the above-mentioned surface modifications, with localized forces (dielectrophoresis, gravity, Brownian motion, suction) is needed to obtain a tight gigaseal for planar patch-clamping.

Acknowledgements

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