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Application Report

Automated Patch Clamp recordings of Gárdos channels (K_{Ca}3.1 / KCNN4) in physiological solutions in human primary Red Blood Cells (RBC)

Recording of the small endogenous Gárdos channel currents in physiological solutions made possible with low-noise amplifiers and consumables with high resistance patch holes (small hole size).

Summary

- Automated Patch Clamp technology enables precise measurement of ion channel activity in human primary Red Blod Cells (RBC).
- This study focuses on characterizing the small endogenous Gárdos channel currents, which are crucial for RBC physiology.
- Recent amplifier improvements, substantially reducing noise, ensured detection of the low endogenous Gárdos currents of human RBC.
- High resistance patch holes (small diameter) are important because they help form stable giga-ohm seals with the cell membrane, especially in delicate and small cells like RBCs.
- Together, these technologies reduce noise and minimise cell damage, resulting in quality and reliable data for understanding ion channel function in RBC.

Introduction

Automated patch clamp technology allows for highly accurate measurement of ion channel activity in human RBC, which is essential for understanding their physiological functions. This study specifically investigates the small endogenous Gárdos channel currents (K_{Ca}3.1 / KCNN4), highlighting their key role in red blood cell physiology. The use of low-noise amplifiers is critical, as it allows recording of low amplitude currents. High resistance patch holes, achieved through small-diameter consumables, facilitate the formation of stable giga-ohm seals with the delicate RBC membranes, reducing cellular damage. The combination of these recent improvements results in reliable, low-noise recordings, thereby supporting robust analysis of ion channel function in RBC.

High resistance (HiR) QPlatess

In automated patch clamp, patch holes with smaller diameter and thereby high resistance (3-7 MΩ) can be highly beneficial. This allows improved giga-ohm seals formations when working with organelles and small cells such as RBC. Thus, this ensures improved accuracy of measurements of ionic currents and membrane potentials.

High resistance, small hole consumables cause less damage to the cell membrane, thus preserving cell integrity during the experiment recordings. The result is improved seal stability over time and reduced electrical noise, leading to clearer data. This is especially important when handling organelles and small cells, which are more delicate and susceptible to damage.

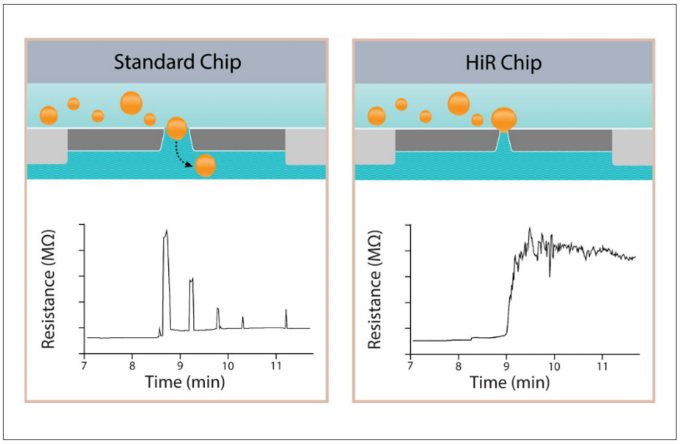

Fig. 1: High-resistance consumables enable capture of and gigaseal formation in small cells. A cross-section illustration of standard (left) and a small-hole, high-resistance (HiR) consumable (right). On a standard chip, cell slipping can be observed on the resistance plot during capture. On a HiR chip this slipping is reduced, and the cell capture can be confirmed by rapid and persistent increase in resistance.

Low noise amplifiers

Low noise amplifiers are crucial when recording small endogenous currents from RBC, as even minor electrical interference can obscure these subtle signals. Minimizing amplifier noise ensures that the detected currents truly reflect channel activity rather than background artifacts. Recent improvements in the QPatch amplifier (Q-amp), including a substantial reduction in amplifier noise, have significantly enhanced the reliability and sensitivity of these measurements. These advancements now make it feasible to extend automated electrophysiology to the level of single-channel recordings, opening new possibilities for detailed ion channel analysis in RBC.

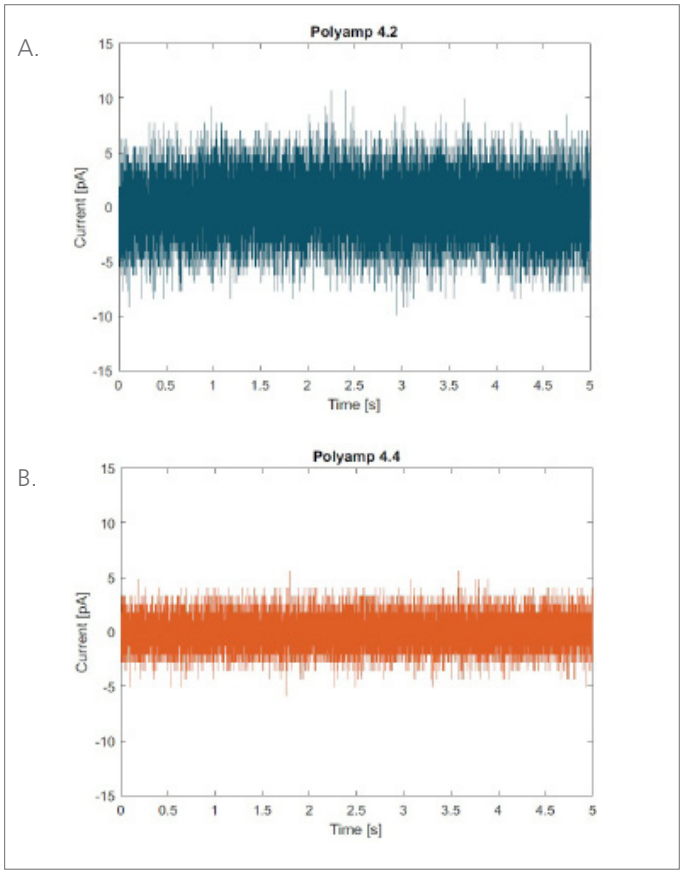

Fig. 2: Noise optimization and performance of the updated QPatch amplifier (Q-amp). Amplifier comparison at 50 kHz sampling, 1 kHz cutoff frequency. **A.** Comparison of current noise: Using an Artificial Reference QPlate (ARQ) in "On-Cell"-Mode, with an artificial membrane resistance of 500 MΩ and holding potential set at 0 mV. The legacy Q-amp (left, blue) exhibits a filtered noise floor of σ = 2.2 pA (50 kHz sampling, 1 kHz Bessel filter cutoff frequency). **B.** Updated Q-amp (right, orange) achieves a 38% reduction in filtered noise (σ = 1.3 pA) and an approximately 50% reduction in unfiltered noise (50 kHz sampling). This improved signal-to-noise ratio enables reliable resolution of current steps as small as 2 pA on an automated platform.

Role and Pathological Significance of Gárdos in RBC

Ion channels in human RBCs play essential roles in maintaining cell volume, membrane integrity, and the ability to traverse narrow microvascular spaces. Among these, the Gárdos channel ($K_{Ca}3.1$ / KCNN4) and the mechanosensitive channel Piezo1 are the two best characterized conductive pathways in RBC membranes and are increasingly recognized as central regulators of RBC physiology and pathophysiology (Larsen *et al*., 1981; Lew *et al*., 2025).

Mechanical activation of Piezo1 triggers Ca^{2+} influx in RBC, which subsequently activates the Ca^{2+}-dependent Gárdos channel, leading to K^+ efflux and cell dehydration. Mutations in KCNN4 and PIEZO1 have been linked to a spectrum of hereditary hemolytic disorders, including hereditary xerocytosis and Gárdos channelopathy (Glogowska *et al*., 2015; Fermo *et al*., 2017).

Furthermore, the Gárdos channel plays a critical role in the dehydration of RBC in sickle cell anaemia. Its activation leads to significant potassium efflux and cellular shrinkage, exacerbating sickling and hemolysis in affected individuals (Lew *et al*., 2005; Rivera *et al*., 2002).

Despite their relevance for disease diagnostics and drug discovery, direct electrophysiological measurements of Gárdos channel activity in primary patient RBCs remain limited. The application of high-throughput Automated Patch Clamp (APC) to these cells offers several advantages: (i) it captures native channel behavior in the physiologically intact membrane, (ii) it enables functional validation of pathogenic mutations without reliance on overexpression models, (iii) it supports pharmacological and mechanistic studies at scale, and (iv) it provides a foundation for personalized therapeutic approaches in rare RBC channelopathies. Together, these capabilities position high-throughput APC as a transformative tool for studying Gárdos channel function and dysfunction directly in primary patient material.

Gárdos channel ($K_{Ca}3.1$ / KCNN4)

Gárdos channel is Ca^{2+}-activated K^+ channel (KCa), which is an integral player in setting the membrane potential and regulating Ca^{2+} homeostasis (Wei *et al*., 2005). Its function is tightly regulated by intracellular Ca^{2+} through a Ca^{2+}-calmodulin-dependent mechanism, rendering the channel largely insensitive to changes in membrane potential. The half-maximal effective concentration (EC_{50}) for Ca^{2+} activation ranges from 95 to 350 nM, closely mirroring the activation profile of other KCa-channels. Ion selectivity of Gárdos follows a type III or IV Eisenmann sequence, with permeability order K^+ (1.0) = Rb^+ > NH^{4+} > Cs^+ >> Na^+, Li^+, $NMDG^+$. The channel exhibits a unitary conductance of approximately 32–39 pS under symmetrical K^+ conditions. The current-voltage relationship is characterized by inward rectification at positive potentials due to voltage-dependent open channel block by intracellular Ca^{2+} and Mg^{2+} ions (Ishii *et al*., 1997; Ledoux et al., 2008). In this study, we develop and evaluate a high-throughput patch clamp assay designed specifically to measure Gárdos channel activity in primary human derived RBCs. By integrating single cell electrophysiology with targeted pharmacological interrogation, we have established a robust framework for studying KCNN4 channel function in health and disease.

Results and discussion

Preparation of RBCs for Automated Patch Clamp

RBCs were obtained from primary patient-derived samples. The cells were isolated by centrifugation, followed by multiple washes using Sophion extracellular solution to ensure removal of plasma and leukocytes. Once purified, the RBCs were prepared for electrophysiological recordings by suspending them in the appropriate medium, ready for automated patch clamp analysis. Careful handling and preparation preserved the physiological integrity of the membrane, allowing for accurate downstream measurements.

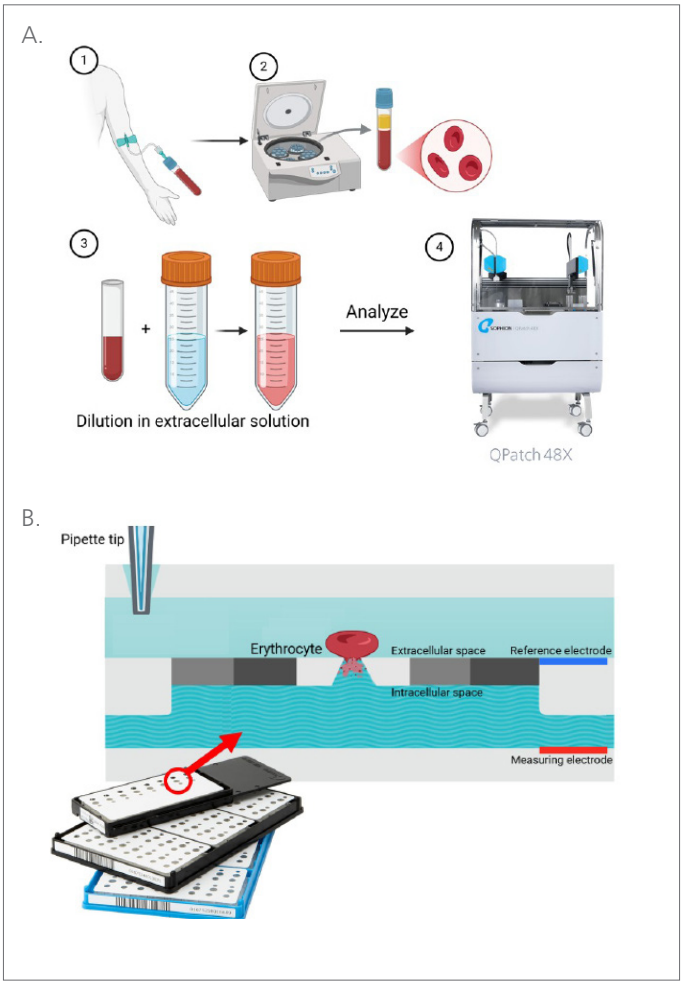

Fig. 3: Methodology for studying ion channel activity in RBCs. **A**. RBCs are isolated via centrifugation and washed with Sophion extracellular solution for electrophysiological recordings. **B**. Patch-clamp setup in one site of 48 sites in consumable: Shear stress is applied to activate Piezo1, and ionic currents are recorded after whole-cell configuration is obtained.

Gárdos in RBCs pespond to pharmacological modulation

This setup enabled us to reliably capture whole-cell potassium currents and systematically evaluate their voltage-dependence through detailed current-voltage (IV) protocols. By applying the pharmacological agents NS309 and TRAM34, it was possible to potentiate and inhibit, respectively, the Gárdos channels in real time (Strøbaek *et al*., 2004; Wulff *et al*., 2000), allowing the pharmacological isolation of the Gárdos-mediated current. Currents sensitive to the inhibitor, TRAM34 were considered Gárdos mediated, and as the baseline current was not significantly different from the background, we used the NS309 potentiated current for further analysis. These experiments provided quantitative insights into both the baseline and modulated activity of the Gárdos channel population.

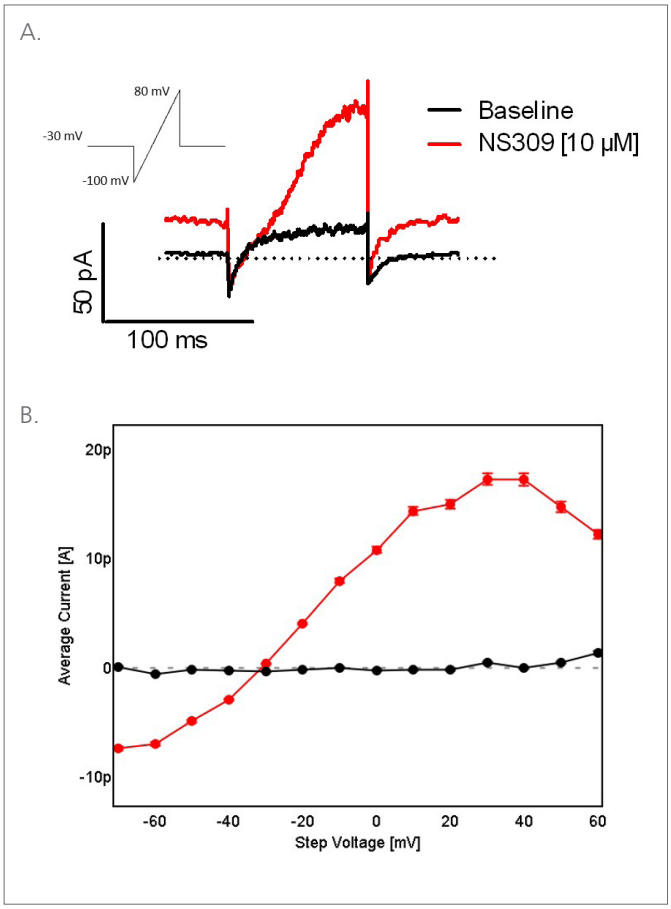

Fig. 4: **A**. Representative current trace recorded from a red blood cell using the voltage protocol shown in the insert. The trace illustrates ionic currents measured under baseline conditions (black) and following application of 10 µM NS309 (red). NS309 application results in a notable increase in current amplitude, reflecting robust activation of Gárdos channels in the cell. **B.** Steady-state current-voltage (IV) relationship in RBC: Whole-cell currents were measured at the end of 1-second voltage steps, each initiated from a resting potential of -30 mV.

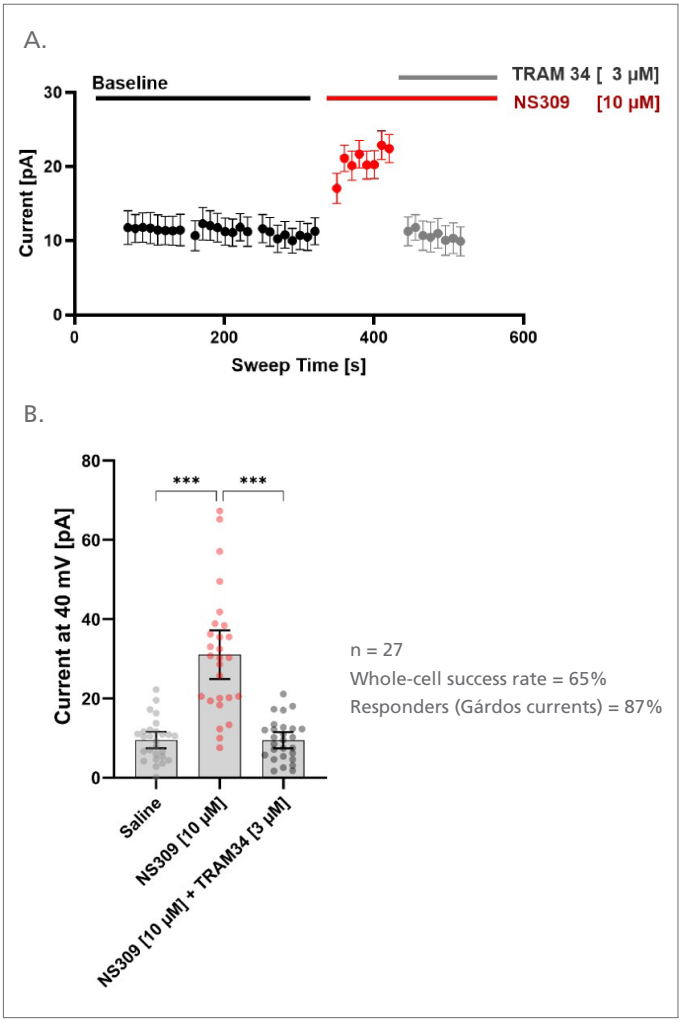

Fig. 5: Electrophysiological characterization of Gárdos activity in RBC (RBCs). **A.** IT plot displaying the current measured at 40 mV from the ramp protocol. Sequential pharmacological modulation: NS309 (10 µM) potentiates the Gárdos channels, followed by inhibition with the selective inhibitor, TRAM34 (3 µM) on the same cell, confirming specific channel activity. **B.** Bar diagram quantifies the current amplitude at 40 mV in a population of RBC under three conditions: First, baseline (all endogenous currents), then potentiation of Gárdos-mediated currents with NS309 followed by inhibition with TRAM34. NS309 significantly increases current amplitude compared to saline, while TRAM34 reverses this effect, returning currents to baseline levels. The Gárdos current was quantified as the TRAM34 sensitive current and found to be 21.6 pA ± 13.8 pA

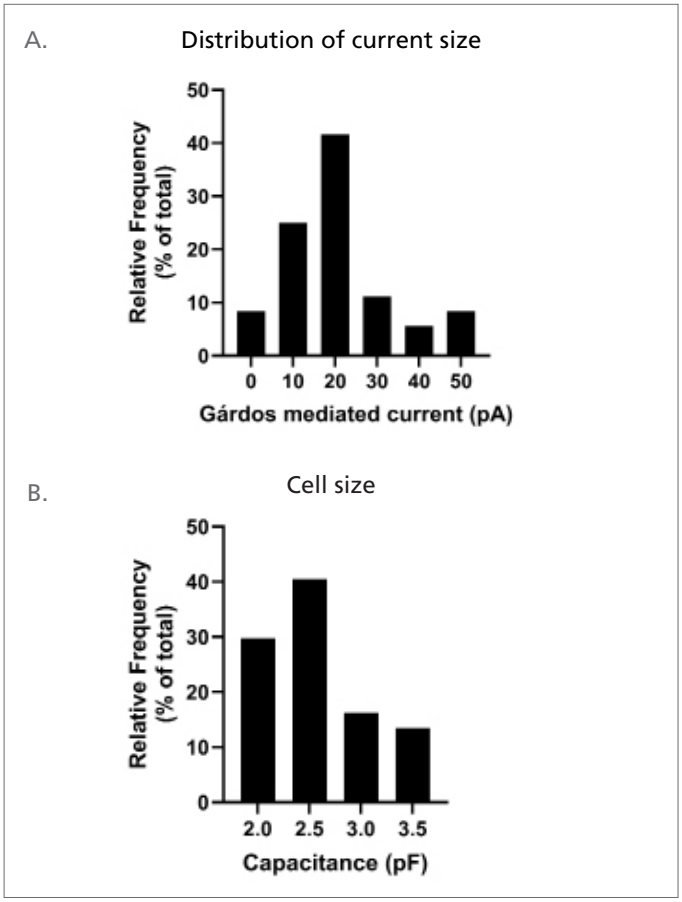

Fig. 6: High-throughput patch-clamp analysis enabled quantification of Gárdos channel current expression and cell size distribution across a heterogeneous red blood cell population: **A.** Histogram displaying the distribution of Gárdos current amplitudes measured across individual RBCs, illustrating the heterogeneity in channel expression within the population. **B.** Histogram depicts the distribution of cell sizes, as determined by membrane capacitance (in pF), providing insight into the variability of RBC-size among the sampled cells. The approach allowed detection of channel activity in both young and aged RBCs, providing robust population-level insights despite the low channel copy number per cell.

Gárdos expression in RBC population innately heterogeneous

The low Gárdos channels copy number on native RBC, makes Gárdos assays in RBCs notoriously difficult. In addition, RBCs lose the nucleus during development and the absence of a translational system causes the Gárdos copy number to significantly decrease throughout the RBC life time, making the population innately heterogeneous (Tiffert *et al.*, 2007). We exploited the throughput of the QPatch to quantify both the current expression (Figure 6A) of this heterogenous population and the distribution in cell size (Figure 6B).

Solutions

For ion channel recordings, two types of solutions were employed. The standard calcium-gated potassium channel recording solution (EC 0.0.1, 1 µM free calcium) was used to robustly detect Gárdos channel activity, yielding a high success rate in patch clamp assays. Additionally, a physiological, seal enhancer–free solution (EC 11.0.1, 400 nM free calcium) was tested. These solutions were critical in supporting high-throughput electrophysiological studies of endogenous ion channel activity in human RBCs.

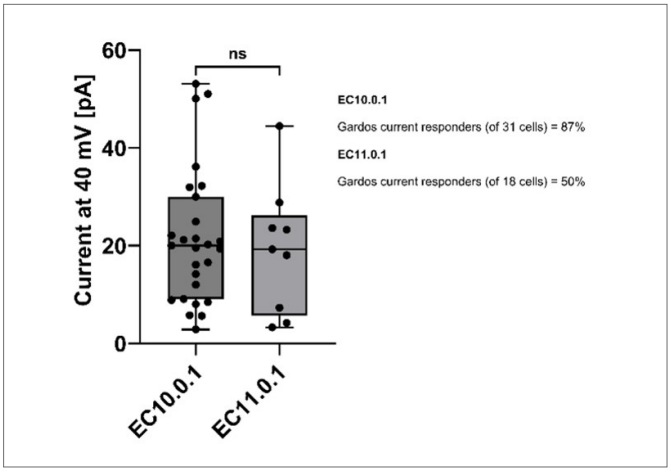

Fig. 7: High-throughput patch-clamp recordings of NS309-induced currents in RBC using different types of solutions. Human RBCs demonstrate similar achieved using the standard calcium-gated potassium channel recording solution (E C10.0.1) and the seal enhancer–free solution (EC 11.0.1). Statistical analysis using student t-test showed no significant difference ($p > 0.05$) between the average current recorded in the standard solution (EC 10.0.1, 21.6 pA ± 13.8 pA) and the physiological solution (EC 11.0.1, 19.1 pA ± 13.2 pA).

Conclusion

High-throughput patch-clamp analysis enables robust characterization of Gárdos channel activity in human RBC. In these experiments, the low-noise amplifiers enabled accurate detection of Gárdos channel activity even at low copy numbers. The use of HiR QPlates facilitated consistent high-resistance seals, which were essential for reliable recordings. The assays reveal significant heterogeneity in channel expression across the RBC population. Both standard and physiological recording solutions yield comparable results, supporting the reproducibility of findings.

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