Automated high throughput patch clamp studies of Piezo1 channel

Sonia Paz[1], ***David Nagy[2]**, Stefania Karatsiompani[3], Jennifer Kefauver[4], Ryan Pak[5], Daniel Sauter[2]

[1] School of Biological Sciences
University of East Anglia, Norwich, United Kingdom

[2] Sophion Bioscience Inc.
213 Burlington Road, Suite 105, Bedford, MA 01730 USA;

[3] Sophion Bioscience A/S
Baltorpvej 154, Denmark, info@sophion, www.sophion.com

[4] University of Geneva
Geneva, Switzerland

[5] Scripps Research Institute
La Jolla, CA

Abstract:

Piezo ion channels convert mechanical stimuli into various biological activities through a process called mechanotransduction. Piezo1 and Piezo2 were identified in 2010 as molecular mediators of the mechanically activated current found across multiple cell types *(Coste et al., 2010)*. While the main function of Piezo2 is the mediation of gentle touch sensation, Piezo1 has functions in numerous physiological processes, including sensing shear stress of blood flow for proper blood vessel development, regulating urinary osmotic pressure, controlling blood pressure and exercise performance *(Faucherre et al., 2014; Li et al., 2014: Martin-Almedina et al., 2018)*. Previous studies have shown that over 25 mutations in Piezo1 cause human disease. However, most mutations have yet to be extensively studied *(Song et al., 2022)*. Piezo1 is activated through cell membrane deformations caused by mechanical forces, such as osmotic pressure, fluid shear stress, substrate stiffness, and confinement. Known stimuli activating Piezo1 in vitro are shear stress, as well as the indentation of a cell with a glass probe and membrane stretching *(Ranade et al., 2014)*.

Summary:

In this study, we aimed to develop an assay to perform Piezo1 recordings on a high throughput automated patch clamp platform. The stimulus applied here was shear stress produced by liquid flow injection containing Yoda1, the Piezo1 agonist, at 26 and 30 ˚C. Fluid flow shear stress is achieved by applying a defined solution flow through the microfluidic recording chamber, creating a uniform shear force for the study's cells. In addition, Yoda 1 slows the inactivation phase of transient currents, enabling better detection of Piezo1-mediated mechanically induced responses *(Syeda et al., 2015)*. HEK293T cells stably expressing mPiezo1-GFP fusion protein and HEK cell line lacking Piezo1were used to develop the assay and ensure the specificity of Piezo1 responses.We demonstrated the feasibility of mechanically stimulating Piezo1 using the high-speed liquid handling feature (or standard speed) in the Qube384 platform using either Yoda1 at 5 µM or 10 µM. Success rate of >80% was achieved using Yoda1 10 µM and shear stress of 5 dyne/cm2 regardless of the temperature (26/30˚C) with an amplitude current around -2 nA. Importantly, no current response was observed in the HEK cell line lacking Piezo1. In addition, we evaluated whether responses could be observed by repeated stimulation. Indeed, a 20-minute wait time between stimulations allowed us to obtain current responses of similar amplitude.

Conclusion:

Our results demonstrate the Qube384 is capable of characterizing Piezo1 in vitro. These assays open the door to a new set of features that allow the identification of antagonists on Piezo1 in the presence of Yoda1 using automated high throughput electrophysiology platforms.

Experimental condition and success rate (SR):

We conducted electrophysiological and mechanical characterization of Piezo1-dependent currents by utilizing the fully automated Qube 384 platform. Piezo 1 overexpressing mPiezo1 HEK293T and Piezo1 knock-out (KO) mHEK293T cell lines were used to develop a robust assay and ensure the specificity of Piezo1 responses. To determine the assay success rates, we assessed the percentage of sites meeting the quality criteria:

1) Membrane resistance: R_{mem} > 200 MΩ
2) Cell capacitance: C_{slow} > 4 pF
3) Minimum peak current: > -200 pA

We conducted assays utilizing the Qube's high-speed liquid dispensing feature (~63 µL/s), which resulted in a shear stress of approximately 5 dyne/cm² on the Qube384 platform. These conditions were applied in conjunction with Yoda1 at concentrations of 5 or 10 µM. To explore the influence of temperature, we conducted recordings at two different temperatures, 26 and 30 °C.

All analysis and figures were prepared in the Sophion Analyzer and Prism 9.3.1 (GraphPad Software, Inc, La Jolla, CA, USA). For detailed composition of the solutions used for this assay, please contact us for further details (info@sophion.com).

Results:

Stimulating Piezo1 through the Qube's high-speed liquid dispensing feature, using Yoda1 at 10 µM to sensitize the channel, is a viable method for generating shear stress and elicit Piezo1 currents. However, applying shear stress alone, without the presence of Yoda1, did not induce any Piezo1-dependent currents. The Piezo1 dependency of this stimulation is confirmed by the lack of elicited currents in the KO cells. The concentration of Yoda1 exhibited a notable influence on the current amplitudes, while they remained unaffected by temperature variations when subjecting the cells to the same stimulation. Additionally, stronger shear stress by the fast liquid dispensing feature of the Qube resulted in larger Piezo1-dependent currents.

Acknowledgement:

We would like to express our sincere gratitude to the Patapoutian Lab at Scripps Research Institute for generously providing us with the mPiezo1 HEK293T and KO mHEK293T cells.

Additionally, we would like to extend our appreciation to Adrienne Dubin and Rose Hill for their invaluable consultations.

Qube 384

- Giga-Ω seal ion channel recordings in 384 cells at once
- A true walk-away screening solution to meet demanding timelines
- Accurate control of temperature, via sensor and feedback loop on the measurement site
- Patented centrifuge for spin down and preparation of cells
- Automatic cell handling
- Individual electrode pairs – no need to ever re-chloride electrodes again

Figure 1: Sophion instrument used for automated patch clamp recordings.

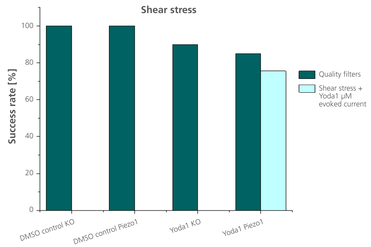

Figure 2: Success rates achieved using Yoda1 at 10 µM in combination with shear stress at 5 dyne/cm2 at 26 ˚C in mPiezo1 HEK293T and in Piezo1 KO mHEK293T cells.

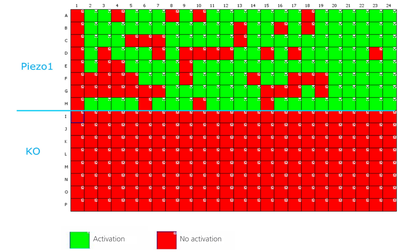

Figure 3: Plate view of a 384 channel QChip.

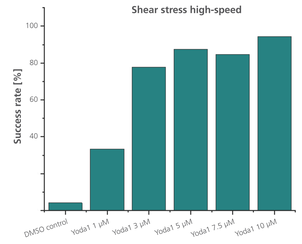

Figure 4: Success rates of activation. The activation of Piezo1 currents is Yoda1 dose-dependent in mPiezo1 HEK293T cells.

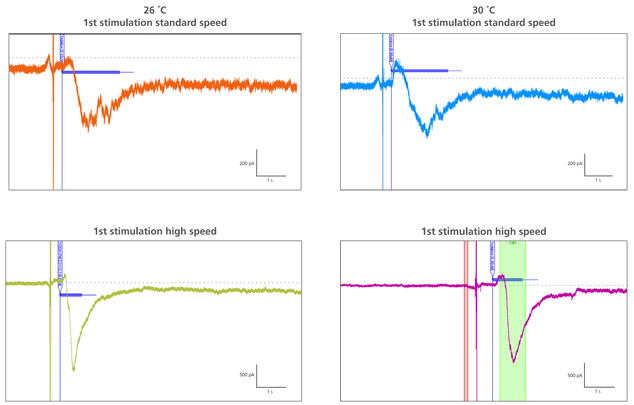

Figure 5: Representative current traces at different temperatures, and shear stress rates using 10 uM of Yoda1.

Project number	Temperature	Speed 1st stimuli	Speed 2nd stimuli	Preincubation time	1st activation SR	2nd activation SR
1038	26	Standard speed	Fast	20 min	Yoda1 10 uM 71.88% - KO cells 0%	**Yoda1 10 uM 67% - KO cells 0%**
1039	26	Fast	Fast	20 min	Yoda1 5 uM 83%/ 10 uM 80.5% - KO cells 0%	Yoda1 5 uM 50% / 10 uM 43% - KO cells 0%
1036	30	Fast	Fast	20 min	Yoda1 10 uM 80.83%	Yoda1 10 uM 36%
1037	30	Fast	Fast	20 min	Yoda1 10 uM 89.58%	Yoda1 10 uM 49.30%
1026	30	Standard speed	Fast	20 min	Yoda1 5 uM 27%/ 10 uM 74.43%	Yoda1 5 uM 62%/ 10 uM 50%
1027	30	Standard speed	Fast	20 min	Yoda1 10 uM 77.17%	**Yoda1 10 uM 56%**
1034	30	Standard speed	Fast	20 min	Yoda1 10 uM 58.71%	Yoda1 10 uM 51.6%

Table 1: Success rates of activation by testing different conditions. The best SR was achieved (*) when the first stimulation was performed by standard dispense speed and the second stimuli was applied with high dispense speed.

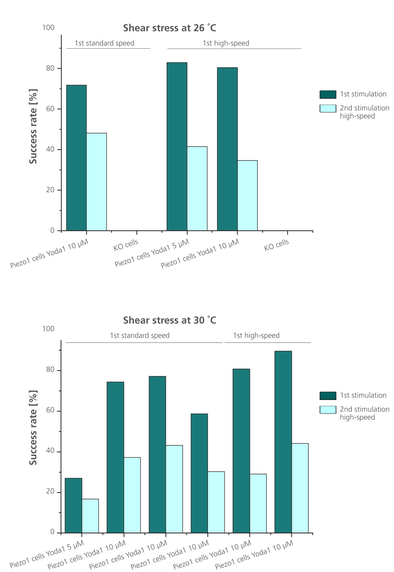

Figure 6: Success rates of activation tested with different liquid dispense speeds and two different temperatures.

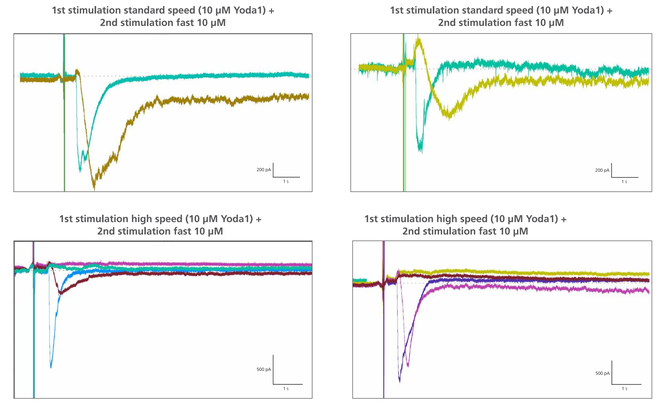

Figure 7: Representative current traces illustrating the double stimulation after 20 minutes recovery time.

References:

Coste, Bertrand, et al. "Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels." Science 330.6000 (2010): 55-60.

Faucherre, Adèle, et al. "Piezo1 plays a role in erythrocyte volume homeostasis." Haematologica 99.1 (2014): 70.

Li, Jing, et al. "Piezo1 integration of vascular architecture with physiological force." Nature 515.7526 (2014): 279-282.

Ranade, Sanjeev S., et al. "Piezo1, a mechanically activated ion channel, is required for vascular development in mice." Proceedings of the National Academy of Sciences 111.28 (2014): 10347-10352.

Syeda, Ruhma, et al. "Chemical activation of the mechanotransduction channel Piezo1." Elife 4 (2015): e07369.

Martin-Almedina, Silvia, Sahar Mansour, and Pia Ostergaard. "Human phenotypes caused by PIEZO1 mutations; one gene, two overlapping phenotypes?." The Journal of Physiology 596.6 (2018): 985-992.

Song, Siqi, et al. "The role of mechanosensitive Piezo1 channel in diseases." Progress in Biophysics and Molecular Biology 172 (2022): 39-49.

Presented at Neuroscience 2023