Mechanical stimulation of PIEZO1 channels using high throughput automated patch clamp

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1 Introduction

PIEZO1 channels are mechanosensitive ion channels that play a pivotal role in sensing mechanical forces in various cell types. Their dysfunction has been associated with numerous pathophysiological states including generalized lymphatic dysplasia, varicose vein disease, dehydrated hereditary stomatocytosis, and malarial resistance. Given its high physiological relevance, investigating PIEZO1 is crucial for the pharmaceutical industry that requires scalable techniques to allow for drug discovery. In this regard, several studies have shown the use of high throughput automated patch clamp (APC) to explore the function and properties of PIEZO1 channels in heterologous expression system as well as primary cells (Rotordam et al., 2018, Parsonage et al., 2022; Karamatic Crew et al., 2023) mainly based on usage of Yoda1, a specific gating modifier of PIEZO1 channels (Syeda et al., 2015). However, to our knowledge, a combination of solely mechanical stimulation and high throughput APC has not yet been available for the study of PIEZO1 channels. Here we show PIEZO1-mediated currents activated by mechanical stimulation (M-Stim) on the SyncroPatch 384.

2 Mechanical stimulation (M-Stim) on the SyncroPatch 384

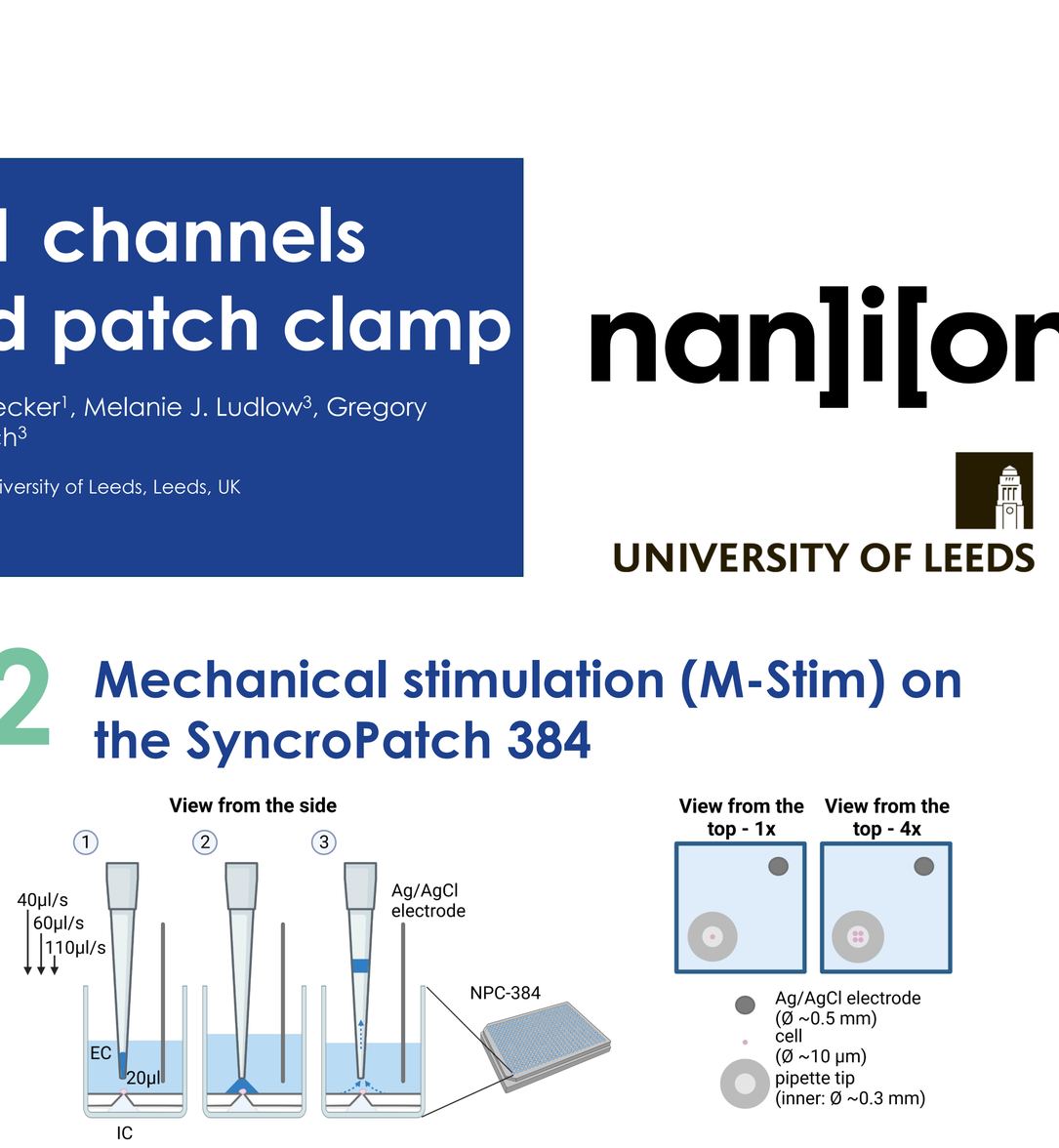

Figure 1: (Left) Schematic illustration of a cross section of one well of an NPC-384 chip. M-Stim delivers a small volume of solution locally to the cell (1 and 2) and uses an aspiration step (3, dashed arrows) to recover the dispensed volume. (Right) Schematic illustration of the top view of one well of an NPC-384 chip with 1 patch hole (1x) and 4 patch holes (4x). Illustrations were created with BioRender.com.

Abbreviations:
n = number of cells for a given experimental condition out of total amount of valid cells
N = number of independent NPC-384 chips
T_{50} = decay time from peak to 50% of the remaining signal

3 Effect of pipetting flow on PIEZO1 responses

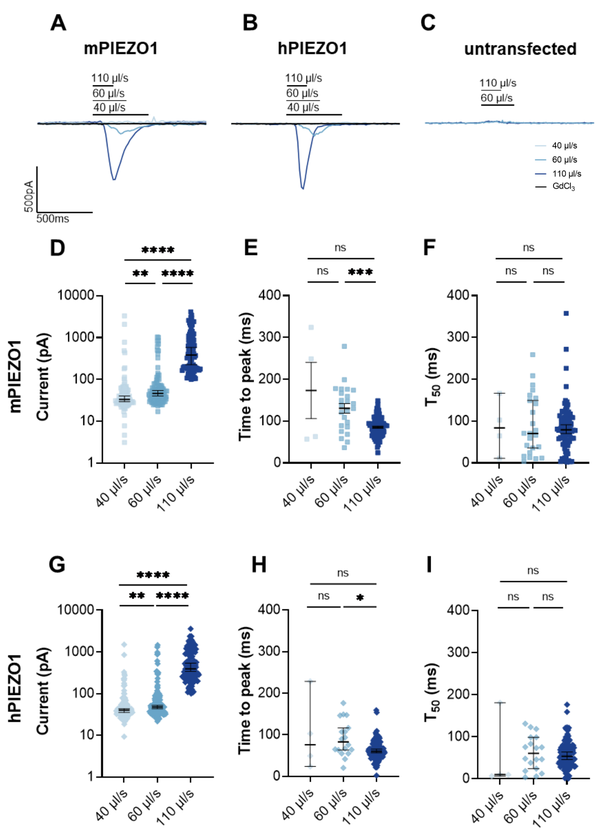

Figure 2: Representative PIEZO1 raw traces of single cells elicited by M-Stim at 40µl/s (light blue trace), 60µl/s (blue trace) and 110µl/s (dark blue trace) and blocked by $GdCl_3$ (black trace) for mPIEZO1 (A), hPIEZO1 (B) and untransfected cells (C). Absolute peak current amplitudes of mPIEZO1 (D) and hPIEZO1 cells (G) plotted against pipetting flow (mPIEZO1: n=123/1440; N=9; **P=0.0073, ****P<0.0001); hPIEZO1: n=123/1471; N=9; **P=0.022, ****P<0.0001). Time to peak and T_{50} values of mPIEZO1 (E-F) and hPIEZO1 cells (H-I) plotted against pipetting flow (mPIEZO1: 40µl/s n=4/1467; 60µl/s n=24/1488; 110µl/s n=88/1440; N=9; hPIEZO1: 40µl/s n=4/1494; 60µl/s n=18/1483; 110µl/s n=103/1471; N=9; ns P>0.05, *P=0.0201,***P=0.0006). All data are shown as values of individual cells with median, and 95% CI indicated in black, tested for statistical significance using a Friedman test (D/G) and Kruskal-Wallis test (E-F/H-I) with Dunn's post-hoc test.

4 Increased current readout using multi-hole chips

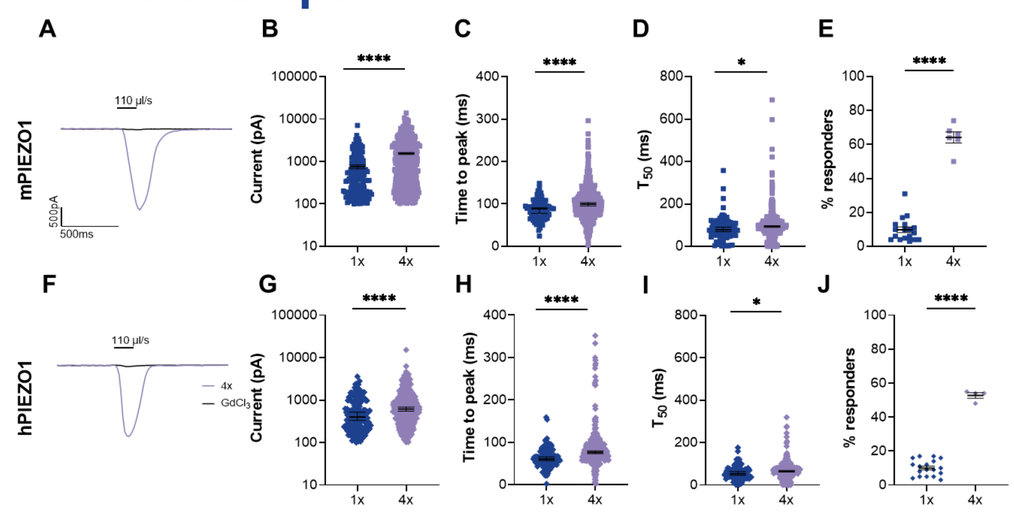

Figure 3: Representative mPIEZO1 (A) and hPIEZO1 (F) raw traces recorded from a 4-hole chip elicited by M-Stim at 110µl/s. Absolute current values of mPIEZO1 (B) and hPIEZO1 cells (G) recorded from single-hole (1x, dark blue) and 4-hole (4x, purple) chips (mPIEZO1: 1x, n=148/1910; N=18; 4x, n=579/941; N=6; hPIEZO1: 1x, n=174/1919; N=18; 4x, n=346/667; N=4; ****P< 0.0001). Time to peak and T_{50} values of mPIEZO1 (C-D) and hPIEZO1 cells (H-I) recorded from 1x (dark blue) and 4x (purple) chips (mPIEZO1: 1x, n=88/1440; N=9; 4x, n=506/843; N=5; *P=0.0211, ****P < 0.0001; hPIEZO1: 1x, n=103/1471; N=9; 4x, n=324/667; N=4; * P=0.0375, ****P < 0.0001). Percentage of mPIEZO1 (E) and hPIEZO1 (J) cells responding to activation by M-Stim at 110µl/s pipetting flow using 1x (dark blue) and 4x (purple) chips (mPIEZO1: 1x, N=18; 4x, N=6; hPIEZO1: 1x, N=18; 4x, N=4; ****P < 0.0001). Data are shown as values of individual cells with median, and 95% CI (B, C, D/G, H, I), or as mean ± SEM (E/J), indicated in black, tested for statistical significance Mann Whitney test and unpaired t-test.

5 Effect of M-Stim combined with Yoda1

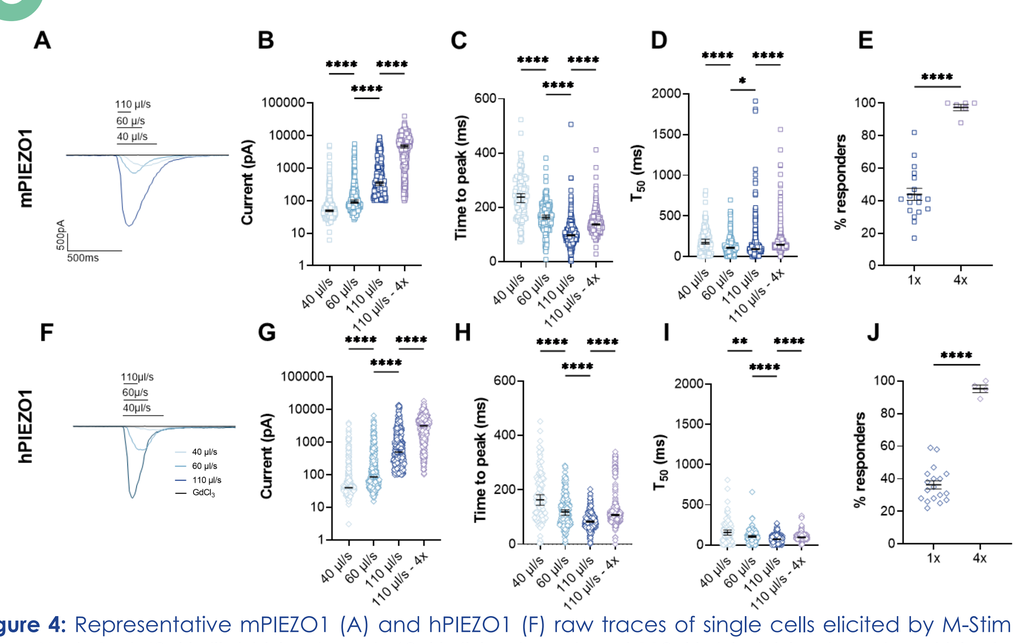

Figure 4: Representative mPIEZO1 (A) and hPIEZO1 (F) raw traces of single cells elicited by M-Stim + 10µM Yoda1 at 40µl/s (light blue trace), 60µl/s (blue trace) and 110µl/s (dark blue trace) and blocked by $GdCl_3$ (black trace). Absolute peak current amplitudes of mPIEZO1 (E) and hPIEZO1 cells (I) plotted against pipetting flow (mPIEZO1: 1x, n=465/1353; N=9; 4x, n=685/722; N=6; hPIEZO1: 1x, n=456/1390; N=9; 4x, n=577/604; N=4; ****P < 0.0001). Time to peak and T_{50} values of mPIEZO1 (C-D) and hPIEZO1 cells (H-I) plotted against pipetting flow (mPIEZO1: 1x, 40µl/s n=142/1420; 60µl/s n=242/1388; 110µl/s n=465/1353; N=9; 4x, 110 µl/s n=662/697; N=5; * P=0.0178, ****P < 0.0001; hPIEZO1: 1x, 40 µl/s n=104/1425; 60 µl/s n=227/1412; 110 µl/s n=460/1390; N=9; 4x, 110 µl/s n=566/604; *P=0.0094, ****P < 0.0001). Percentage of mPIEZO1 (E) and hPIEZO1 (J) cells responding to M-Stim + Yoda1 at 110µl/s pipetting flow recorded from 1x (dark blue) and 4x (purple) chips (mPIEZO1: 1x, N=18; 4x, N=6; hPIEZO1: 1x, N=18; 4x, N=4, ****P < 0.0001). Data are shown as values of individual cells with median, and 95% CI (B, C, D/G, H ,I), or as mean ± SEM (E/J), indicated in black, tested for statistical significance using Kruskal-Wallis test with Dunn's post-hoc test and unpaired t-test.

6 Summary

- Fast pipetting flow (110 µl/s) is a key factor for the mechanical stimulation (M-Stim) of PIEZO1 channels on the SyncroPatch 384
- Activation of hPIEZO1 by M-Stim results in faster signal onset and faster signal decay compared to mPIEZO1
- Using M-Stim with multi-hole (4x) chips increases signal amplitude and number of responding wells, and represents a valid tool for the study of PIEZO1 channels on the SyncroPatch384
- M-Stim can be combined with chemical stimulation by e.g. Yoda1 to maximize the success rate (i.e., number of responding cells)
- M-Stim provides a highly parallelized, objectified approach to allow for mechanically induced PIEZO1 activation, enabling mutants' investigations and compound screening, with the ultimate goal of accelerating the development of new medical strategies.

References

Rotordam MG et al. (2018). Blood, 132, 1031.

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Karamatic Crew V et al. (2023). Blood, The Journal of the American Society of Hematology, 141(2), 135-146.

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