Single-cell optical action potential measurement in human induced pluripotent stem cell-derived cardiomyocytes

Supplemental material

Seibertz F, Reynolds M, Voigt N

Native murine cardiomyocyte preparation for voltage imaging

Protocols for murine cardiomyocyte optical voltage investigation are largely identical to those outlined in the main text for human iPSC-CMs. Differences of note include the entirety of the cellular preparations outlined in section 1.1. Murine cardiomyocytes are not cultured, instead they are mechanically and enzymatically isolated from excised hearts as previously described.[1] FluoVolt loading concentration remains the same as listed, however, loading protocol 2.3–2.7 proceeds under alternative directions. A visual guide is displayed in **Figure 2A.**

Mouse cardiomyocyte isolation yields a pellet suspended in 0.2 mM Ca^{2+} storage solution in a 15 ml falcon tube. Decant this solution to 2 ml and add 2 µl 0.1X Fluovolt loading solution (Contents provided step 2.2), and 5 µM blebbistatin (optional). Loading proceeds in low light conditions for 30 minutes at room temperature. After, aspirate all supernatant and resuspend the cardiomyocytes with 2 ml fresh, warm Tyrode's solution (Contents provided in step 2.1). An identical bath chamber (Setup detailed in step 2.5) is utilised however the rectangular coverslip is coated with 2 µl of 1 mg/mL laminin prior to direct application of 500 µl cell suspension. This is to limit cardiomyocyte movement during perfusion.

References:

1. Voigt, N., Zhou, X.-B., Dobrev, D. Isolation of human atrial myocytes for simultaneous measurements of Ca^{2+} transients and membrane currents. *Journal of visualized experiments : JoVE*. (77), e50235–e50235, doi: 10.3791/50235 (2013).