Establishing reference conditions for electrophysiological recordings of spontaneously beating neonatal rat cardiomyocytes on a multi-electrode array

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Background: Multi-electrode arrays (MEA) provide a useful tool for non-invasive mapping of cardiac electrophysiology in both cardiomyocyte monolayer cultures and atrial/ventricular tissue slices. The use of microelectrodes embedded into glass plates allows for extracellular electrophysiological recordings across cell monolayers on successive days without the potentially long term damaging effects of voltage sensitive fluorophores. Neonatal rat ventricular myocytes (NRVMs) represent a useful model for assessing inter-cardiomyocyte conduction properties as they can be maintained in culture, spontaneously beating as a functional monolayer. Comprehensive methodological details for investigating conduction properties in NRVM cultures on MEA glass chips are limited. In particular, there are difficulties in optimally plating these myocytes on the glass plates, such that NRVMs are confluent, spontaneously beating and maintaining sufficient contact with the recording electrodes. The aim of this study was to optimise the plating conditions for NRVMs on MEA glass chips and assess the relative benefits of recording field potentials on MEA chips coated with the conductive polymer "Pedot" (MCS, Reutlingen, Germany), customized to reduce electrode impedance.

Methods: Neonatal rats (1-2 day old) were anesthetised in 80% ethanol and decapitated. Their hearts were then excised and enzymatically digested. NRVMs were seeded onto MEA chips coated in fibronectin and cultured in Dulbecco's modified eagle medium at varying cell densities on both regular and Pedot coated MEA chips (60EcoMEA and 60PedotEcoMEA, MCS). MEA chips included 59 recording electrodes of 100 μm dimeter, arranged within a grid with inter-electrode distance of 700 μm. At 5-6 days culture, spontaneous beating rate (SBR), conduction velocity and field potential amplitude (FPA) were recorded at 37ºC on a 2100 MEA head stage using Cardio2D software (MCS, Reutlingen, Germany). Optimal electrophysiological recordings were determined by comparing cell culture plating methodologies ('droplet' *vs* whole-plate culture) and MEA surface properties (standard *vs* Pedot coated). Results are presented as mean ± SEM. Comparisons between groups were performed with a Student's unpaired t-test. Differences were considered significant at $P<0.05$.

Results: Plating cells as a 'droplet' concentrated over the recording electrode grid greatly increased the capacity to measure field potentials across all recording electrodes (average 99.6% electrode coverage across 5 chips) compared with those cells plated across the entire MEA chip (average 27.1% electrode coverage across 3 chips). The SBR was significantly slower in 'droplet' plated NRVM monolayers compared with those monolayers covering the entire MEA chip ('droplet' *vs* entire chip, bpm; 91 ± 4 *vs* 207 ± 21, n=4-10, $P<0.05$), with a mean inter-myocyte conduction velocity of 23.76 ± 1.09 cm/s. The use of a 'Pedot' coating on the MEA glass plates dramatically increased field potential amplitudes compared with non-coated plates (μV; 2796 ± 331.9 *vs* 467.5 ± 36.17, n=6, $P<0.05$), with no change in SBR and conduction velocity. Critically, SBR, conduction velocity and field potential amplitudes did not change over a 30 minute recording period, indicating preparation stability.

Conclusion: This study highlights the importance of plating conditions and MEA chip characteristics for obtaining optimal field potentials from NRVM monolayers on the MEA. Restricting the plating of NRVM cultures across only the recording electrode grid improved field potential morphology and cell beating/conduction parameters. The use of 'Pedot' coated chips greatly improved field potential amplitude and would therefore comprise the preferred MEA electrode composition in culture setting. Together, these conditions ensure a stable preparation over a period of at least 30mins, suitable for short-term conduction mapping studies with acute pharmacological treatments. These studies provide baseline conditions and recording signal characterization for use as reference in future investigations of conduction abnormality and arrhythmogenicity.