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Optimising cardiomyocyte monolayer culture methodology for high-spatial resolution electrophysiological mapping

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Introduction: Multielectrode arrays (MEAs) are a useful tool for non-invasive, high-spatial resolution mapping of extracellular electrophysiology in cardiomyocyte monolayers. Neonatal rat ventricular myocytes (NRVMs) provide a good model for assessing intercardiomyocyte conduction as they can be maintained spontaneously beating in culture as an electrically-coupled monolayer. Comprehensive methodological details for culturing NRVMs onto glass MEAs are required. The aim of this study was to optimise plating conditions for NRVMs onto glass MEAs, and to maximise cardiomyocyte-electrode adherence and field potential recording capacity on adapted MEAs coated with the conductive polymer "Pedot".

Method: NRVMs were isolated from neonatal Sprague-Dawley rats then seeded at varying densities onto fibronectin-coated glass MEAs (60EcoMEA or 60PedotEcoMEA, Multichannel Systems; electrode spacing 700μm, diameter 100μm, 8x8 matrix) and maintained in culture. 5-6 days after isolation, optimal culture conditions were determined by recording field potentials and generating activation maps to compare; plating technique (central cell droplet vs whole-MEA culture) and MEA surface properties (uncoated vs Pedot-coated), using a MEA2100 system. Culture responsiveness to β-adrenergic stimulation (1μM isoproterenol) was also assessed. Comparisons between groups were performed with *t*-tests or one-way ANOVA, as appropriate. Differences were considered significant at $P<0.05$.

Results: Restricting culture of cardiomyocytes to the central recording matrix enhanced total signal detection capacity from 27.1% to 99.6%, allowing consistent measurement of rapid conduction velocities (mean conduction velocity: 23.8±1.1cm/s). Use of Pedot-coated MEAs significantly increased field potential amplitude (uncoated *vs* Pedot-coated: 0.47±0.036mV *vs* 2.8±0.33mV; $n=6$, $P<0.05$). In optimised cultures, 1μM isoproterenol significantly increased the spontaneous beating rate (control *vs* isoproterenol-treated: 90±6bpm *vs* 138±14bpm; $n=4$, $P<0.05$) and decreased field potential duration (control *vs* isoproterenol-treated: 155.1±9.1ms *vs*105.6±8.8ms; $n=4$, $P<0.05$). This was associated with a small, but significant increase in conduction velocity (control *vs* isoproterenol-treated: 22.3±1.7cm/s *vs* 24.4±1.5cm/s; $n=4$, $P<0.05$), validating the culture's responsiveness to positive chronotropic stimulation.

Conclusion: This study highlights the importance of confining the plating of cell monolayers to the central recording matrix on the MEA, in addition to demonstrating the advantages of using Pedot-coated MEAs. Optimised cultures have been validated through demonstrating positive canonical responsiveness to β-adrenergic stimulation. This study provides baseline conditions and recording signal characterisation for use as reference in future investigations of conduction abnormality and arrhythmogenicity.