Tracing neural development and rhythms in a benchtop perfusion platform for combined long-term microelectrode in vitro electrophysiology and time-lapse imaging

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Most *in vitro* electrophysiology studies extract information and draw conclusions from representative, temporally limited snapshot experiments. This approach bears the risk of missing decisive moments that may make a difference in our understanding of physiological and metabolic events. We presenton a simple benchtop cell culture perfusion system adapted to commercial microelectrode arrays (MEAs), multichannel electrophysiology equipment, and common inverted microscopy stages for simultaneous and uninterrupted extracellular electrophysiology and time-lapse imaging at ambient CO2 levels. The concept relies on a transparent, replica casted polydimethylsiloxane (PDMS) perfusion cap, gravity- or syringe-pump-driven perfusion, and pre-conditioning of pH-buffered serum-free cell culture medium to ambient CO2 levels at physiological temperatures. The low-cost microfluidic in vitro enabling platform, which allows to image cultures immediately after cell plating, is easy to reproduce and adaptable to the geometries of different cell culture containers. It permits the continuous and simultaneous multimodal long-term acquisition or manipulation of optical and electrophysiological parameter sets, thereby considerably widening the range of experimental possibilities. Continuous extracellular recordings over a period of up to 70 days revealed details on both sudden and gradual neural activity changes in maturing cell ensembles with large intra-day fluctuations. Correlated time-lapse imaging unveiled rather static macroscopic network architectures with previously unreported local morphological oscillations on the timescale of minutes.