Human induced pluripotent stem cell-derived neuronal models for in vitro neurotoxicity testing

Anke M. Tukker | Fiona M.J. Wijnolts | Remco H.S. Westerink

Neurotoxicology Research Group, Institute for Risk Assessment Sciences (IRAS), Faculty of Veterinary Medicine, Utrecht University, Box 80.177, NL-3508 TD, Utrecht, The Netherlands; r.westerink@uu.nl

Introduction

Current neurotoxicity testing still relies heavily on ethically debated, expensive and time consuming animal experiments. As a result, there is a clear need for innovative high-throughput in vitro test strategies. However, these tests receive considerable criticism suggesting that they are too simplified to mimic the in vivo brain and are therefore not predictive enough. Neurotoxicity models should thus contain a functional neuronal network consisting of a mixed population of excitatory and inhibitory neurons. Recently, human induced pluripotent stem cell (hiPSC)-derived neurons became available. However, they are not as well characterized as the current gold standard, i.e. primary rat cortical cultures.

Aim

• Demonstrate the mixed neuronal nature of hiPSC-derived (co-)cultures.
• Assess spontaneous network activity and bursting behavior of hiPSC-derived neuronal (co-)cultures containing different ratios of inhibitory and excitatory neurons.
• Explore applicability of hiPSC-derived neuronal (co-)cultures for high-throughput animal-free neurotoxicity testing.

Materials and Methods

Cell culture: hiPSC (human induced pluripotent stem cells) were derived from skin biopsies and cultured as described previously1. hiPSCs were differentiated into neurons and astrocytes according to the manufacturer’s protocol. Neuronal cultures were maintained in DMEM/F12, astrocytes in DMEM and a mixture of hiPSC-derived neurons and astrocytes was cultured in Neurobasal media, supplemented with B27 and FGF. To culture neuronal (co-)cultures, hiPSC-derived neurons and astrocytes were (co-)cultured in line with manufacturer’s protocols as described in Table 1.

IF-staining: Cells were fixed at DIV7, 14 and 21 and labeled with (III)Tubulin (neurons), DAPI (nuclei) and/or S100β (astrocytes), as described previously1 and visualized using a Leica SPEII confocal microscope.

mwMEA: Neuronal activity was measured at DIV4-21 using multi-well micro-electrode arrays (mwMEAs; Axion Biosystems Inc.) as described previously2 and in Figure 1. Following a baseline measurement at DIV21, cultures were exposed and effects on spontaneous neuronal activity and bursting behavior were measured.

Results

Development of spontaneous activity and bursting

Table 1. Composition and density of different hiPSC-derived neuronal models.

<table>
<thead>
<tr>
<th>Type of culture</th>
<th>Cell types and ratio</th>
<th>Seeding density/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCell® Glutaneuron monoculture</td>
<td>100% Glutaneurons</td>
<td>100,000</td>
</tr>
<tr>
<td>iCell® Glutaneuron-Astrocyte co-culture</td>
<td>50% Glutaneurons/50% Astrocytes</td>
<td>150,000</td>
</tr>
<tr>
<td>iCell® Glutaneuron-iCell® neuron-Astrocyte co-culture</td>
<td>50% Glutaneurons/15% iCell® neurons/35% Astrocytes</td>
<td>120,000</td>
</tr>
</tbody>
</table>

Figure 2. Immunofluorescent images of iCell® Glutaneuron monoculture (A), iCell® Glutaneuron-Astrocyte co-culture (B) and iCell® Glutaneuron-iCell® neuron-Astrocyte co-culture (C) illustrating development of network structures with neuronal (III)Tubulin (green) and, when applicable, glial markers (S100β; red). Nuclei are stained with DAPI (blue).

Figure 3. Spike raster plots of different hiPSC-derived cultures. Each row depicts one electrode, each tick mark represents one spike of a 150 s interval (A) illustrating the pattern of activity of the iCell® Glutaneuron monoculture (A), the iCell® Glutaneuron-Astrocyte co-culture (B) and the iCell® Glutaneuron-iCell® neuron-Astrocyte co-culture (C) at DIV7 (left), DIV14 (middle) and DIV21 (right). The cumulative trace (purple box) above each plot depicts the population spike time histogram indicating the synchronized activity between the different electrodes (network burst).

Figure 4. MSR (spikes/sec; bars) and % spiking wells (line; A) and MBR (bursts/min; bars) and % bursting wells (line; B). Mean ± SEM from 39-54 wells (n = MSR) and n = 4-12 (MBR) for iCell® Glutaneuron monoculture (left), n = 38-42 (MSR) and n = 2-37 for the iCell® Glutaneuron-Astrocyte co-culture (middle), n = 37-46 (MSR) and n = 6-37 (MBR) for the iCell® Glutaneuron-iCell® neuron-Astrocyte co-culture (right).

Conclusions and Discussion

• hiPSC-derived models form neuronal structures with different neuronal subtypes.
• All cultures develop spontaneous network activity and bursting, but activity is more synchronized in the presence of astrocytes.
• Co-cultures can be modulated with seizurogenic compounds and neurotoxicants.
• Ratio of inhibitory and excitatory neurons may influence chemical sensitivity.
• hiPSC-derived models appear amenable as a prioritization tool for neurotoxicity testing.