PrimeSurface™ Plate 96U application

Stimulus effects Evaluation

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For detailed information refer to:
Stimulus effects of extremely low-frequency electric field exposure on calcium oscillations in a human cortical spheroid. Saito A, Shiina T, Sekiba Y Bioelectromagnetics. 2025;46:e22521.

Background

To accurately evaluate the biological effects of electromagnetic fields (EMFs) at the cellular level, it is important to use biological samples that closely resemble human organs in experimental studies. However, previous studies have not conducted EMF exposure experiments using cultured neuronal networks derived from the human brain and central nervous system. In this study, human cortical spheroids (hCSs), composed of neuronal cells derived from human induced pluripotent stem cells (hiPSCs) and possessing three-dimensional neuronal network structures, were applied to EMF exposure experiments. The suitability of hCSs for evaluating stimulus effects, which serve as the foundation of international EMF exposure guidelines, was investigated. hCSs, produced using PrimeSurface™, were demonstrated to mimic three-dimensional neuronal network structures in vivo and were deemed appropriate for assessing neuronal stimulation responses in vitro. This application note describes the method for producing hCSs using PrimeSurface™ and highlights their utility as an innovative in vitro evaluation model for studying neuronal stimulation effects induced by EMFs.

Human Cortical Spheroids (hCS) Preparation

hiPSC-derived neural progenitor cells (ReproNeuro; ReproCell) were seeded onto PrimeSurface™ 96U plates under the following conditions.
Cell density: $3x10^4$ cells/well
Differentiation medium: ReproNeuro MQ culture solution (medium change at 3, 7, and 14 days)
Long-term culture: NPB medium based on the B-27 Plus Neuronal Culture System (Thermo Fisher Scientific) from Day 21
hCS was generated following several months of culture on PrimeSurface™ 96U plates.

Evaluation of Functional and Stimulus Effects Using hCS

Functional evaluation of hCS using a multi-electrode array (MEA) recording system: Using the MEA system (MED64) from Alpha MED Scientific Inc., extracellular potentials changes associated with maturation were recorded from the same hCS and evaluated over several months.

Evaluation of stimulus-induced responses caused by EMF exposure using Ca imaging: Changes in neural network activity during exposure to ELF-EF were evaluated using Ca imaging with a fluorescence microscope. For the EMF exposure experiments, hCS samples with diameters in the range of 0.9-1.1 mm were used to ensure uniform sample size. The fluorescent indicator Fluo-8 AM (AAT BioQuest) was used for Ca imaging.

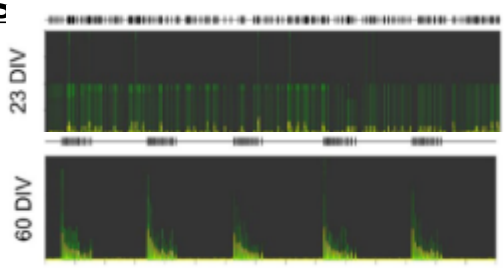

Figure 1

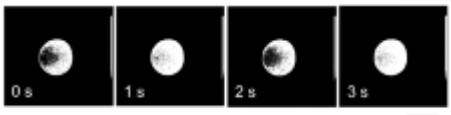

Figure 2

PrimeSurface™ is a great tool for efficiently producing spheroids of uniform size.

Results

- As a result of evaluating the extracellular potential around the hCS over time, it was found that stable burst-like neural network activity could be detected after approximately 2 months of culture (Figure 1).
- Using Ca imaging, neural network activity patterns consistent with extracellular potentials were optically detected (Figure 2).
- During ELF-EF exposure, the occurrence patterns of neural network activity changed significantly compared to non-exposed (Control) conditions (Figure 3).
- These results indicate that responses induced by ELF-EF exposure can also be detected using hCS.

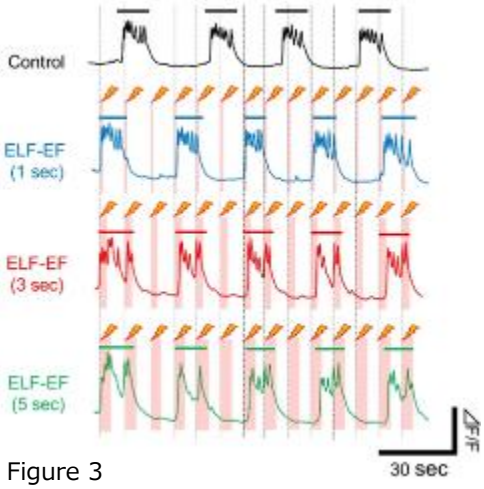

Figure 3

3D hCS generated using PrimeSurface™ are useful for evaluating the stimulus effects of electromagnetic field induced under environments that more closely resemble in vivo conditions.

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