iPSC-DERIVED NEURONAL CULTURES IN 3DPROSEED™ HYDROGEL WELL PLATE

Ectica Technologies AG, Zurich, Switzerland

Contact: info@ectica-technologies.com

Summary

We describe the use of 3DProSeed™ well plate for the simple and automation-compatible establishment of iPSC-derived neuronal cultures in synthetic 3D hydrogel. iPSC-derived neurons seeded on the hydrogel surface gradient rapidly protrude neurites in depth to form 3D networks. Neurons were viable for at least 20 days and showed spontaneous calcium activity. We propose a novel hydrogel platform for the development of assays for neurodegeneration and neurotoxicology in 3D, providing simplicity in use, the highest level of automation compatibility and the possibility to establish co-culture systems by sequential seeding.

Introduction

Hydrogels are widely used as an artificial extracellular matrix to grow neural cells in a three-dimensional (3D) environment. 3D cultures have the advantage of closely recapitulating aspects of the human tissues including the architecture and organization among cells (cell-cell and cell-matrix interactions) and more physiologically relevant diffusion characteristics. In 2014, Kim and co-workers reported in Nature a human neuronal cell culture 3D model in Matrigel [1] demonstrating an increased physiological relevance of the protein expression profile compared to standard 2D cultures.

Culturing cells within hydrogels normally requires their encapsulation in the hydrogel during its formation [2]. The encapsulation process is required because of the very limited penetration in 3D of cells deposited on the surface of a pre-casted gel. The encapsulation process reduces the throughput of the assay, increases the variability and necessitates the simultaneous seeding of different cell populations in case a co-culture assay is envisaged. To tackle these important limitations we developed hydrogels with an in-depth surface density gradient promoting the infiltration in 3D of cells deposited on the hydrogel surface (3DProSeed hydrogels) [3]. Here we describe the establishment of iPSC-derived neuronal culture in 3DProSeed, with the long term goal of establishing automation-compatible 3D models for neurodegenerative and neurotoxicology screenings.

Materials and methods

3DProSeed™ hydrogel well plate. The 3DProSeed™ hydrogel plate consists of a 96-well black microtiter plate with 180 micron glass bottom, containing a fully synthetic pre-assembled and hydrated hydrogel for ready-to-use and automation-compatible cell-based assays. The key innovation is the hydrogel surface featuring a so-called "in depth-density gradient" which enables the penetration into the hydrogel bulk of cells deposited on it. The hydrogel is a poly(ethylene glycol)-based formulation containing cell adhesion (RGD sequences) and degradation (MMP-cleavable sequences) motives.

Cells and media. Human iPSC-derived neurons (CNS.4U™) were kindly offered by Axiogenesis AG (Cologne, Germany). Neurons were cultured in a supplemented Neurobasal medium composition provided by Axiogenesis AG.

Cell seeding procedure. The storage buffer was removed from the wells and 200µL of cell suspension containing 4.0×10^4 iPS-derived neurons was added to each well.

Immunostaining and calcium imaging. Samples were fixed and stained for tubulin (TUJ1, 1 : 300 in 3% BSA in PBS, Sigma-Aldrich, cat. no. T2200). TUJ1 was stained overnight at 4 °C and rinsed three times in one hour with PBS followed by 4 hour incubation with an Alexa Fluor 488 Goat anti-rabbit IgG (1:200 in PBS, abcam, cat. no. ab150113).

To obtain an optical readout of intracellular calcium fluctuations indicative of neural activity, neurons were transduced with a neuron-specific AAV GCaMP6 vector (AAV1.Syn.GCaMP6f.WPRE.SV40; Penn Vector Core,

USA) after 10 days at a 1:1000 dilution by volume. Culture medium was replaced fully 5 days after the addition of the viral vector. Neurons where imaged at 17 DIV with an epifluorescent microscope acquiring image every 100 ms for assessing the transient calcium.Inspection of the cell cultures was performed with a Leica SP5 confocal microscope equipped with long distance 10x and 63x objectives. Image treatment and 3D reconstructions was performed with Imaris.

Results and discussion

Seeding of iPSC-derived neurons on 3DProSeed hydrogels. The neurons were seeded on the hydrogel surfaces, cultured over two weeks and stained for TUJ1. Figure 1 shows a 3D reconstruction of the network of neurites growing into the 3D hydrogel.

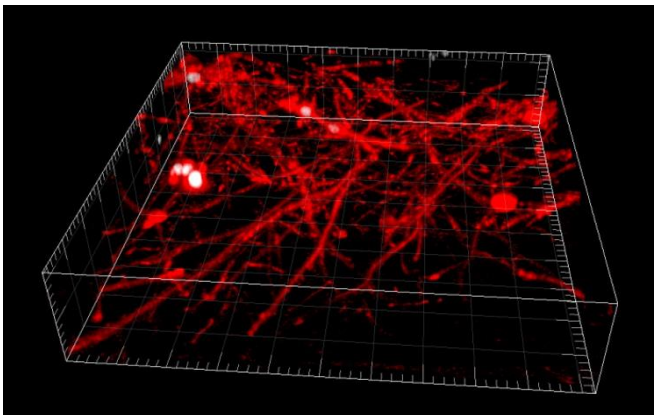

Figure 1. 3D reconstruction showing neurons growing into the hydrogel. Red indicates TUJ1 staining. Major tick size 150 µm. Video of a Z-stack can be found here: https://drive.google.com/file/d/0B7RRpzC9FRHocGxIb0ITckxEUkE/view?usp=sharing.

Calcium imaging. Figure 2 represents a time-lapse of neurons transfected with virus vector carrying genes for protein calcium sensor GCaMP showing spontaneous activity at day 19.

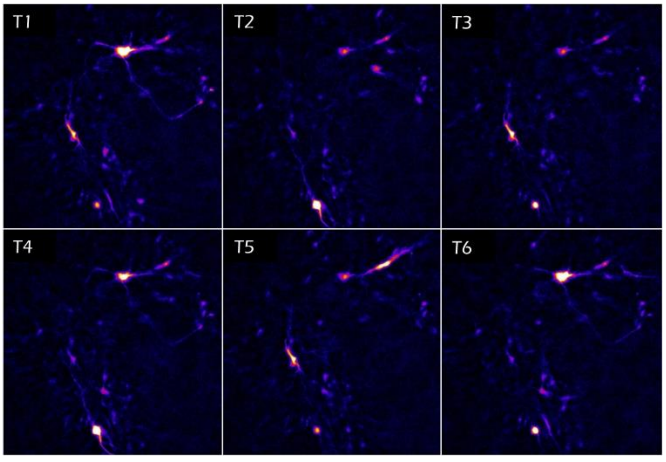

Figure 2. iPSC-derived neurons cultured in 3DProSeed show spontaneous calcium activity 19 days after seeding. Video of time-lapse can be found here: https://drive.google.com/file/d/0B7RRpzC9FRHoN1RhQ2E2UGszdk0/view?usp=sharing.

Compatibility with high-content analysers for automated neurotoxicology screenings.

We tested the compatibility with the Molecular Devices ImageXpress® Micro Confocal High-Content Imaging System. A small panel of known neurotoxic agents were tested on these cultures. Information such as neuron count, neurite length and branching was extracted to verify toxicity of the molecules. More information can be found here:

https://drive.google.com/file/d/1edOcaixn8ofCxaFGuc4Sgj0DX9axY_Fm/view?usp=sharing

Conclusion and outlook

This note explains the use of 3DProSeed™ well plate for the simple and automation-compatible establishment of iPSC-derived neuronal cultures in 3D synthetic hydrogel. Neurons were viable for at least 20 days, developed dense 3D networks of neurites and showed spontaneous calcium activity. In future application notes we will show the establishment of co-cultures by sequentially seeding astrocytes once the neuron culture has been established and also the differentiation of iPSCs towards neurons in the gel and the subsequent addition of glia cells.

Acknowledgement

Ectica Technologies AG thanks Axiogenesis AG for providing the proprietary iPSC-derived neurons and related culture media with supplements.

Ectica Technologies AG thanks the Greta Thompson-Steckel and Prof. János Vörös at the Laboratory of Biosensors and Bioelectronics of ETH Zurich and University of Zurich for supporting the experimental work.

Literature

[1] Se Hoon Choi, Young Hye Kim, et al. , Nature 515, 274–278 , 2014.

[2] Steven R Caliari & Jason A Burdick, Nature Methods 13, 405–414 , 2016

[3] Mllleret V., Simona B.R. et al. Healthc. Mater. 2014 & PCT/EP2012/076426