Xeno-Free Dual Coating Models for Neuronal Activity

In vitro models may be used to study spontaneous neuronal activity (SNA) by culturing iPSCs under xeno-free materials that best replicate the physiological environment. Advanced BioMatrix's (ABM) high purity, xeno-free reagents effectively support functional neuronal activity assays that provide a good understanding of neuronal function, response to their environment, and connectivity with other cells. The SNA of iPSC-derived neurons and astrocytes were assessed using ABM's xeno-free dual coatings, and extracellular matrix combined with Laminin 511.

Materials to Consider	Catalog No.	Starting Concentration
Poly-D-Lysine (PDL)	5049	100 µg/mL
Poly-L-Ornithine (PLO)	5058	100 µg/mL
Fibronectin (FTN)	5050	50 µg/mL
Vitronectin (VTN)	5051	50 µg/mL
Laminin 511 (LN)	5344	3.3 µg/mL

Strategy: Co-Culture on Dual Coatings

Coat one of PDL, PLO, FTN, VTN with LN and co-culture neurons with astrocytes

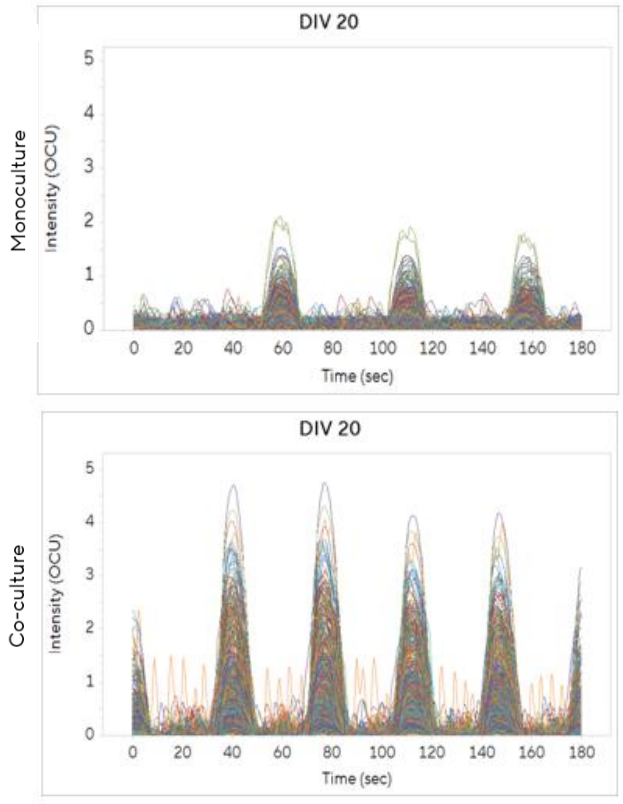

Figure 1. Synchronous SNA in Mono and Co-Cultures at 20 Days in vitro.

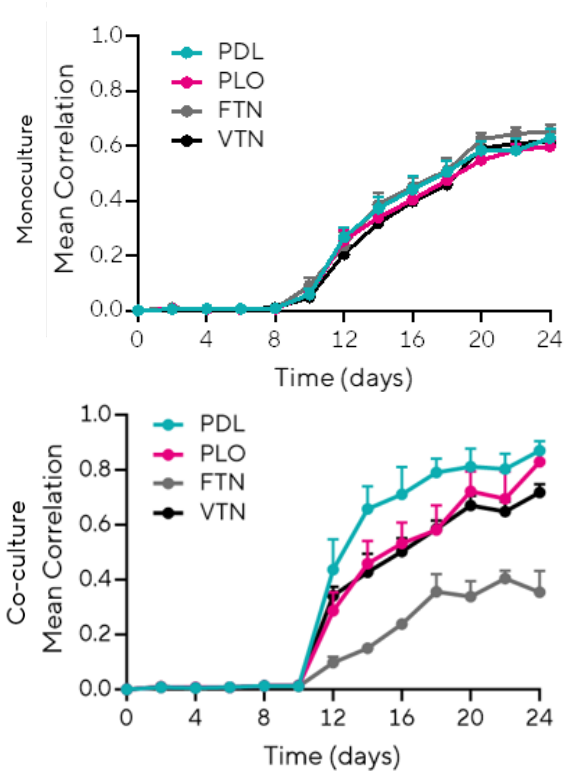

Figure 2. Increasing trend of SNA with ABM's xeno-free coatings.

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Xeno-Free Dual Coatings Support Neuronal Activity Models

Abstract

The following study was conducted to assess neuronal activity of human iPSC-derived neurons and astrocytes in Advanced Biomatrix's (ABM) xeno-free coating materials consisting of a xeno-free extracellular matrix listed below and Laminin 511. Calcium oscillations were measured to determine correlations in spontaneous neuronal activity (SNA). The study demonstrated that these advanced models in ABM's xeno-free materials successfully support derived iPSCs' functional ability to form excitatory connections.

Table 1. Plate coating matrices for neuronal assays.

Materials	Supplier	Catalog. No.	Final Concentration
Poly-D-Lysine (PDL)	Advanced Biomatrix	5049	100 µg/mL
Poly-L-Ornithine (PLO)	Advanced Biomatrix	5058	100 µg/mL
Fibronectin (FTN)	Advanced Biomatrix	5050	50 µg/mL
Vitronectin (VTN)	Advanced Biomatrix	5051	50 µg/mL
Laminin 511 (LN)	Advanced Biomatrix	5344	3.3 µg/mL

Introduction

Culturing iPSCs under conditions that best replicate the physiological environment is essential to support neuronal outgrowth, adherence, and dynamic cellular functions. Neuronal activity assays provide advanced understanding of how neurons function, respond to their environment, and connect with each other. This study investigated spontaneous neuronal activity (SNA) to further understand the effect of ABMS' xeno-free coatings plus Laminin 511 on the ability of neurons to form excitatory connections. ABM's solutions were used to dual-coat tissue culture plates where iPSC-derived glutamatergic neurons were seeded with or without iPSC-derived astrocytes. Neuronal activity was monitored every 24 hours using a neuronal specific genetically encoded calcium indicator (GECI).

Results

ABM's Xeno-free coatings support functional neuronal activity of iPSC-derived neurons and astrocytes

Representative 3-minute calcium traces for monocultures and co-cultures are shown, reflecting changes in fluorescence intensity for active cells in each well for all ABM coatings (Figure 3A). The fluorescence intensity was measured over time to create calcium traces, showing spontaneous neuronal activity at different stages. At 9 days in vitro (DIV), the activity was random and asynchronous, with small amplitude differences. By 20 DIV, synchronized activity with larger amplitude was observed, indicating neuronal connectivity. Co-cultures showed more synchronized activity than monocultures. Quantification of correlation over time showed that co-

cultures exhibited synchronized calcium bursts about 2 days earlier than monocultures at 12 DIV (Figure 3B). There were no significant differences between coatings for monocultures, but for co-cultures, the strongest correlation was seen with PDL. These results suggest that xeno-free coatings support iPSC-derived glutamatergic neuronal activity.

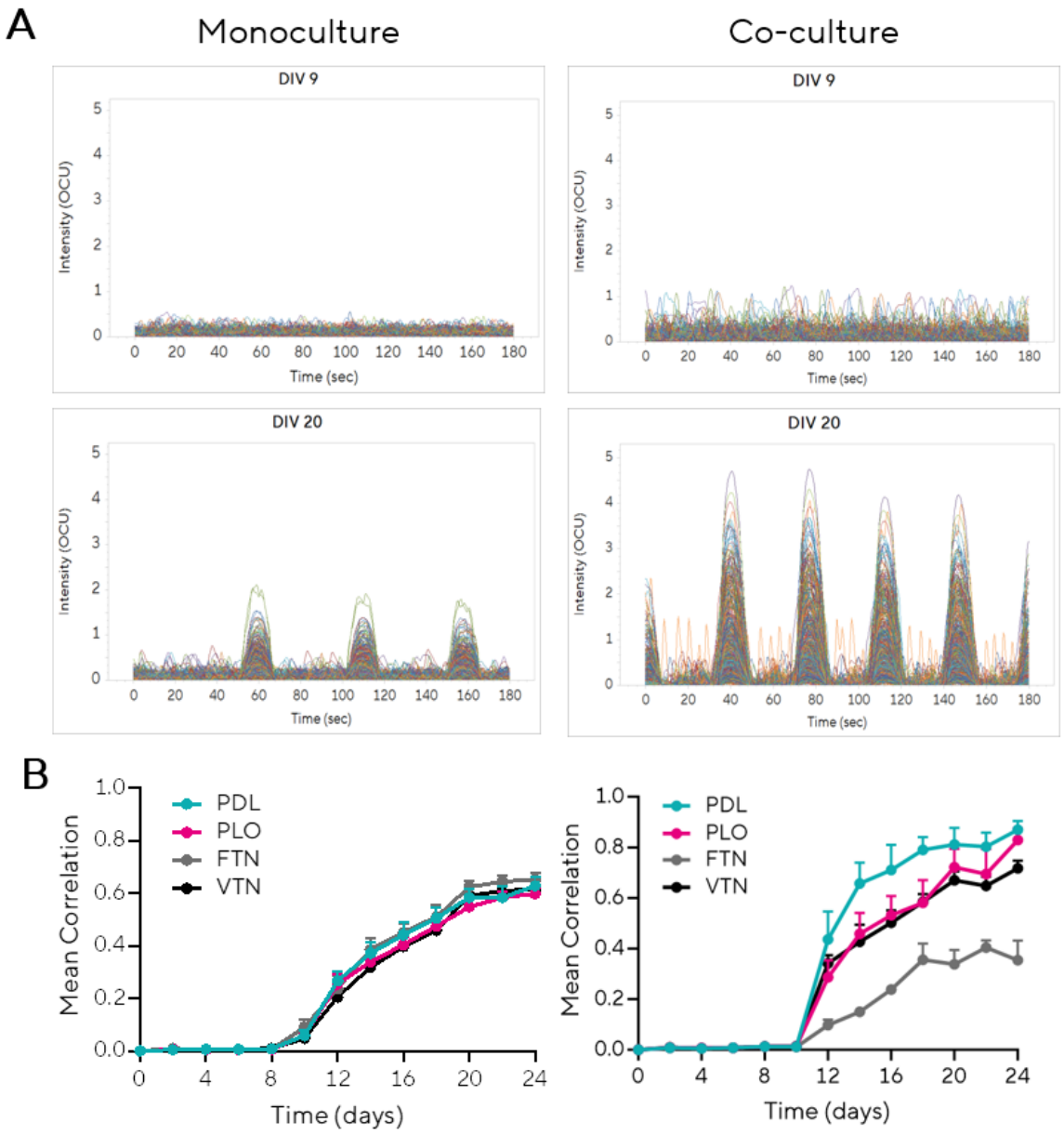

Figure 3. Neuronal activity of iPSC-derived glutamatergic neurons in monocultures and co-cultures with astrocytes on ABM's xeno-free coatings. (A) Asynchronous (DIV 9) and synchronous (DIV 20) spontaneous neuronal activity (SNA) in all ABM coatings + Laminin 511. (B) The mean correlation in SNA of ABM coatings + Laminin 511 (data represents mean ± SEM).

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Conclusion

In summary, this study showed that iPSC-derived neurons and astrocytes are successfully co-cultured on ABM's xeno-free biomatrices, which support neuronal activity. The neurite activity tests revealed that the neurons form synaptic connections and respond to environmental changes. Most importantly, the successful culture and activity of these co-cultures on xeno-free coatings demonstrate a high-quality, clinically relevant model for studying neurological diseases and for future translational applications.

Methods and Materials

iPSC-derived glutamatergic neurons were seeded on dual-coated 96-well plates with or without iPSC-derived astrocytes for co-cultures and monocultures, respectively. The neurons were then infected with neuroburst, and short-term calcium-flux kinetics were captured over 24 days.

Cell Culture & Plate Coating

96-well plates were coated with solutions from ABM at the indicated concentrations (Table 1). Each xeno-free solution was added (100 µL/well) and left to adhere overnight at room temperature. Plates were then rinsed 4X with sterile water and left to dry completely (~1 hour). For dual coatings, Laminin 511 was then added on top of the base coating and incubated at 37°C for 1 hour and removed just before seeding cells. iPSC-derived glutamatergic neurons and astrocytes were processed following the manufacturer's recommendations and maintained in complete BrainPhys or astrocyte medium, respectively (Table 2).

Table 2. Material required for cell culture and assays.

Materials	Supplier	Cat. No.
96-well Flat-bottom Plate	TPP	92096
Cell Culture Grade Water	Corning™	25-005-CM
iCell® Glutaneurons, 01279	Fujifim Cellular Dynamics	C1060
iCell® Astrocytes 2.0, 01279	Fujifim Cellular Dynamics	C1249
BrainPhys™ Neuronal Medium	Stem Cell Technologies	5790
iCell® Nervous System Supplement	Fujifim Cellular Dynamics	M1031
iCell® Neural Supplement B	Fujifim Cellular Dynamics	M1029
iCell® Astrocytes 2.0 Medium	Fujifim Cellular Dynamics	M1048
N-2 Supplement	ThermoFisher	17502-048
5-Fluoro-2′-deoxyuridine	Sigma	F0503
Uridine	Sigma	U3003
L-Glutamic Acid, monosodium salt monohydrate	Sigma	G2834
Kainate monosodium	Sigma	K0250

Incucyte® Neurolight Orange Lentivirus	Sartorius	4808
Incucyte® Neuroburst Orange Lentivirus	Sartorius	4736

Neuronal Activity Assay

The neuronal activity assay measured spontaneous neuronal activity in live cells using the Incucyte® Neuroburst Orange Lentivirus (neuroburst), a genetically encoded calcium indicator (GECI). 96-well plates were coated using the methods stated. iPSC-derived glutamatergic neurons in BrainPhys media were added to the pre-coated plates at 40,000 cells/well for monocultures and 20,000 cells/well for co-cultures. For co-cultures, iPSC-derived astrocytes were added 2 hours after plating the neurons. After 2 days, the media was replaced with neuroburst lentivirus in complete BrainPhys medium (100 µL/well). After 16-24 hours, the lentivirus and media were removed, and fresh media containing FdU/U was added. Plates were monitored daily for 25 days using the Incucyte® Live-Cell Analysis System, with 50% media changes every day. The system captured fluorescent images every 3 minutes, and calcium oscillations were analysed using the Incucyte® Neuronal Activity Analysis Software.

Statistical analyses were performed using the Incucyte® Software and reported as mean ± standard error of mean (SEM). Graphs were produced using GraphPad Prism 10.

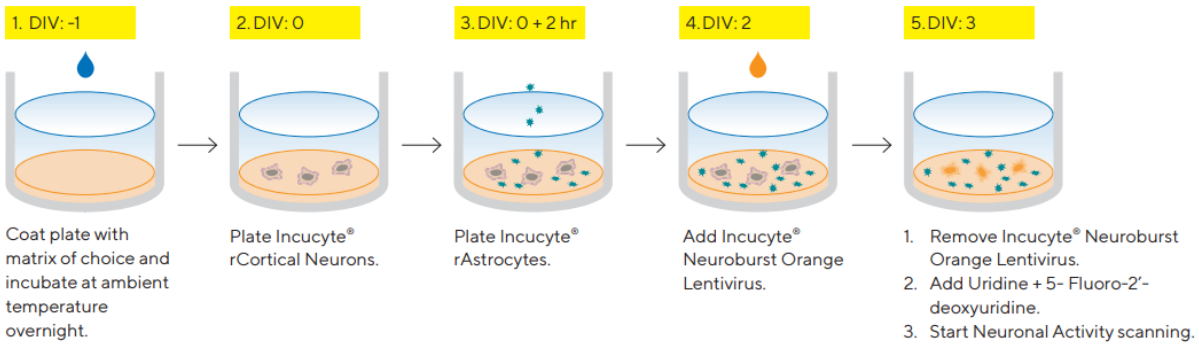

Figure 4. Workflow of neuronal activity assay.

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