ADVANCED BIOMATRIX
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APPLICATION NOTE

Xeno-Free Models for Neurotoxicity Assays

Current methods for culturing iPSCs are hindered by components that may contain traces of xeno contaminants that affect cell behavior, lead to inaccurate results, raise immunological concerns, and limit their use in translational applications. *In vitro* models using Advanced BioMatrix's (ABM) xeno-free materials may be used to study neuronal behavior by culturing iPSCs under conditions that best replicate their physiological environment. ABM's high purity, xeno-free reagents effectively support neuronal excitotoxicity assays that ensure the retention of cellular behavior. The excitotoxic response of iPSC-derived neurons and astrocytes were assessed using ABM's xeno-free dual coating consisting of Poly-D-Lysine and Laminin 511.

Materials to Consider	Catalog No.	Starting Concentration
Poly-D-Lysine (PDL)	5049	100 µg/mL
Laminin 511 (LN)	5344	3.3 µg/mL

Advanced BioMatrix's PDL and LN coating successfully supported the culture of iPSC-derived neurons and co-culture with astrocytes and retained iPSC's excitotoxic behavior. Monocultures and co-cultures were treated with Glutamate, a main excitatory neurotransmitter, and kainate, a neuroexcitatory agonist. ABM's xeno-free biomatrices provide a more accurate and clinically relevant model for studying neurophysiology and neurological disorders.

Strategy: Co-Culture on Dual Coating

Coat PDL with LN 511 and co-culture neurons with astrocytes

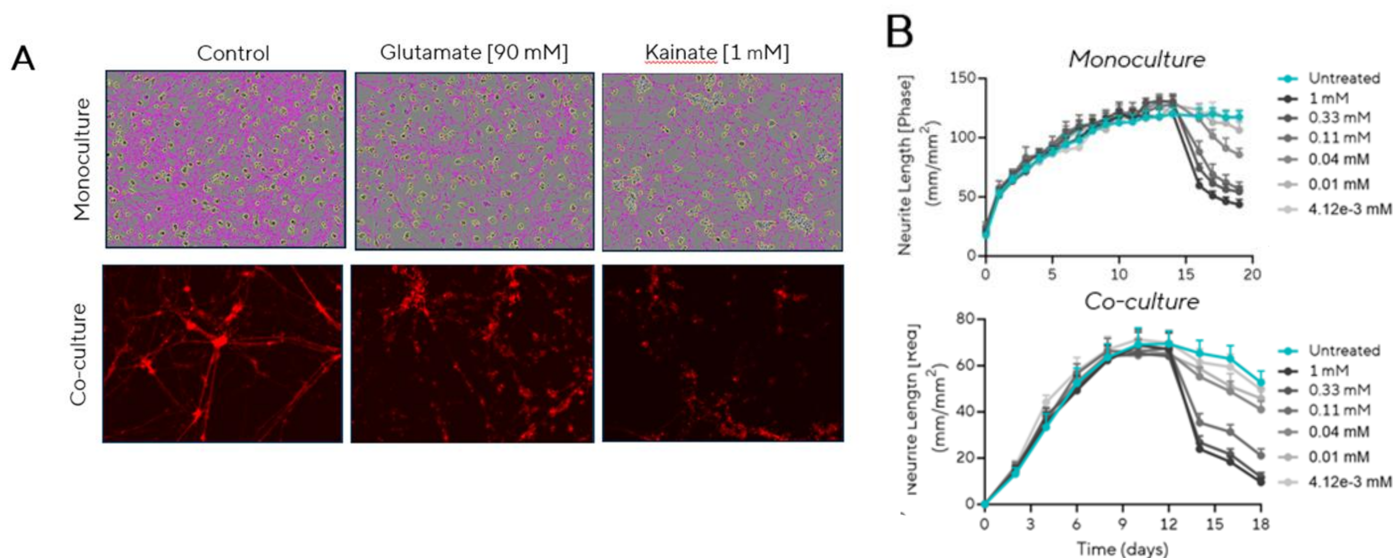

Figure 1. Excitotoxic treatment of iPSC-derived neurons co-cultured with astrocytes on Advanced Biomatrix xeno-free coatings. (A) Neurite outgrowth at 19 days in vitro (post-treatment). (B) Neurite length during culture stabilization and following treatment with kainate.

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Xeno-Free Dual Coatings Support Neurotoxicity Assays

Abstract

The following study investigated the response of human iPSC-derived neurons and astrocytes to excitotoxic stimuli when cultured in Advanced Biomatrix's (ABM) xeno-free Poly-D-Lysine (PDL) and Laminin 511. Neurite outgrowth was monitored over time. Results showed that neurite outgrowth decreased in a concentration- and time-dependent manner in both monocultures (iPSC-derived neurons) and co-cultures (neurons and astrocytes), reflecting the natural excitotoxic response and creating accurate neuronal disease models.

Table 1. Xeno-free matrices for dual coating.

Materials	Supplier	Catalog. No.	Final Concentration
Poly-D-Lysine (PDL)	Advanced Biomatrix	5049	100 µg/mL
Laminin 511 (LN)	Advanced Biomatrix	5344	3.3 µg/mL

Introduction

Current methods for culturing both iPSCs and neurons are limited by components that may contain traces of xenogenic contaminants, which can affect cell behavior and lead to inaccurate results.[1] These contaminants also raise immunological concerns, limiting their use in translational applications.[2] In this study, excitotoxicity assays were performed on iPSC-derived glutamatergic neurons, both in monoculture and co-culture with iPSC-derived astrocytes, to ensure these disease models retain functional behavior when using ABM's xeno-free solutions. Glutamate, a main excitatory neurotransmitter, or kainate, a potent neuroexcitatory agonist,[3] was added at various concentrations to induce excitotoxicity, which occurs when nerve cells are damaged or die due to overstimulation by excessive excitatory neurotransmitters. Neurite length was used as a marker to assess neurotoxicity.[4,5]

Results

ABM's Xeno-free coatings support excitotoxicity studies in iPSC-derived neuron monocultures and co-cultures

Representative phase images of iPSC-derived glutamatergic neurons in monoculture on PDL and Laminin 511 show differences in cells (yellow) and neurites (pink) following treatment with either glutamate or kainate after 19 days in vitro (DIV), just 5 days post-treatment. Similarly, fluorescent images of neurons co-cultured with iPSC-derived astrocytes on PDL and Laminin 511 shows induced neurite retraction and cell death (red) at 19 DIV for both glutamate and kainate (Figure 1A). Quantification illustrates neurite lengths were drastically reduced over time (Figure 1B), demonstrating a concentration- and time-dependent decrease in response to kainate with EC50 values of 44.7 and 74.1 for monoculture and co-culture respectively (Figure 1C). A similar treatment response was observed with glutamate in both monoculture and co-culture experiments (data not shown).

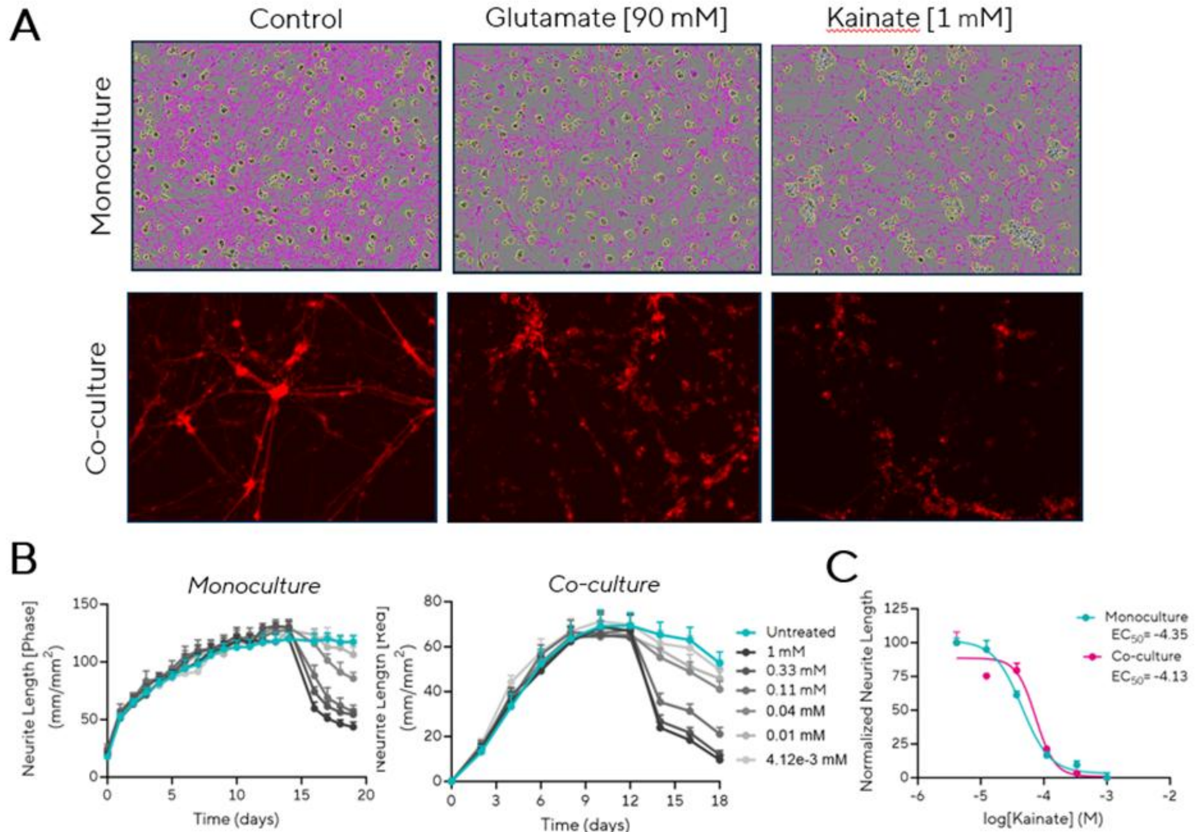

Figure 1. Excitotoxic compound treatment of iPSC-derived neurons monoculture and co-cultures with astrocytes on Advanced Biomatrix xeno-free PDL and LN. (A) Neurite outgrowth at 19 DIV (post-treatment) for each condition. (B) Neurite length during culture stabilization and following treatment with kainate. (C) Concentration-response curves for normalized neurite lengths in the presence of kainate at day 17 (data represents mean ± SEM).

Conclusion

In summary, this study showed successful co-culture of iPSC-derived neurons and astrocytes in ABM's xeno-free biomatrices, creating more accurate models for neuronal research. When treated with glutamate or kainate, the expected excitotoxicity response was seen when neurons and astrocytes were cultured in ABM's xeno-free PDL and Laminin 511. The combination of xeno-free coatings and co-culture of iPSCs provides a clinically relevant model for studying neurological diseases, enhancing future translational applications and addressing the need for optimized research in human neurophysiology and disorders.

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Methods and Materials

Cell Culture & Plate Coating

Monocultures or co-cultures with iPSC-derived neurons and astrocytes were seeded on plates coated with Poly-D-Lysine and Laminin 511 solutions for 14 days before treating with glutamate or kainate. 96-well plates were coated with 100 µL PDL from ABM at a 100 µg/mL concentration and incubated overnight at room temperature. Plates were then rinsed 4X with sterile water and left to dry completely (~1 hour), according to ABM's directions for use. Laminin 511 was then added 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 1).

Table 1. Materials required for cell culture and assays.

Materials	Supplier	Cat. No.
96-well Flat-bottom Plate	TPP	92096
Poly-D-Lysine	Advanced BioMatrix	5049
Laminin 511	Advanced BioMatrix	5344
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

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Neurite Outgrowth and Excitotoxicity Assay

As shown in Figure 2, iPSC-derived glutamatergic neurons were seeded into pre-coated plates at 15,000 cells/well, allowed to settle for 20 minutes, and incubated for 2-3 hours for proper adherence. For co-cultures only, Incucyte® Neurolight Orange Lentivirus in complete BrainPhys medium was added to the plate (100 µL/well) and incubated for 16-24 hours before replacement with fresh media. iPSC-derived human astrocytes were then added at 15,000 astrocytes/well. Approximately 48 hours post-plating astrocytes, 5-Fluoro-2'deoxyuridine and uridine (FdU/U) were added to stop astrocyte proliferation. Both monocultures and co-cultures were maintained with complete BrainPhys media (200 µL/well) with 50% media replacement every other day. An Incucyte® Live-Cell Analysis System was utilized for image acquisition and analysis. HD Phase images were acquired every 6 hours and analyzed using integrated Incucyte® Neurotrack Analysis Software.

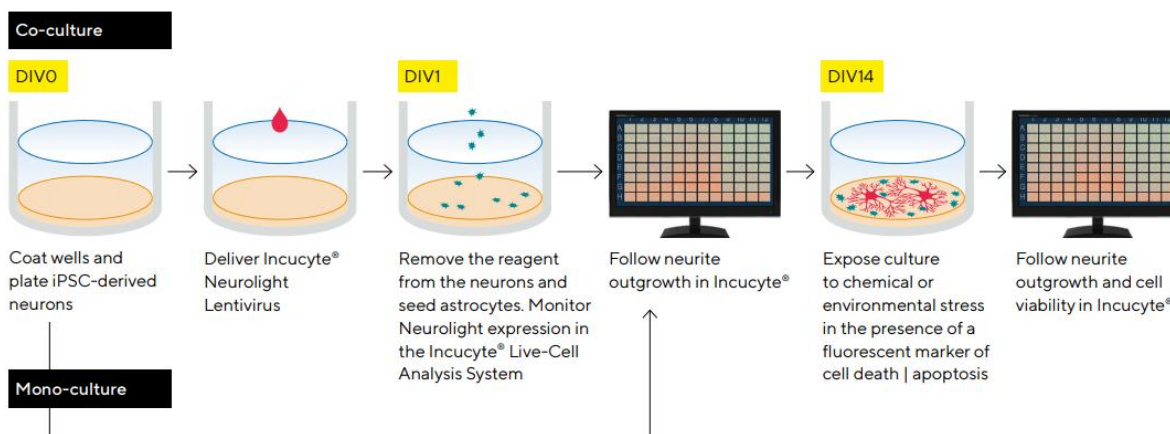

Figure 2. Workflow of neurite outgrowth and excitotoxicity assay.

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