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APPLICATION NOTE

Improved Neuronal Outgrowth Models

Neurological conditions are the leading cause of illness and disability worldwide, affecting more than 3 billion people.[1] *In vitro* models may be used to study these neurological conditions; however, many available components may include xeno-contaminants, which can affect cellular behavior or cause iPSCs to differentiate into other lineages, leading to inaccurate results. High-purity, xeno-free reagents from Advanced BioMatrix may be utilized to more accurately investigate neuronal dynamics in conditions that more closely resemble physiological environments.

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

Advanced BioMatrix's xeno-free solutions have been tested and demonstrated improved neurite outgrowth in dual coatings (figure 1). These have also effectively supported co-cultures of neurons and astrocytes showing an increase in neurite length over time (figure 2).

Strategy #1: Dual Coat

Coat with LN and one of:
PDL, PLO, FTN, VTN

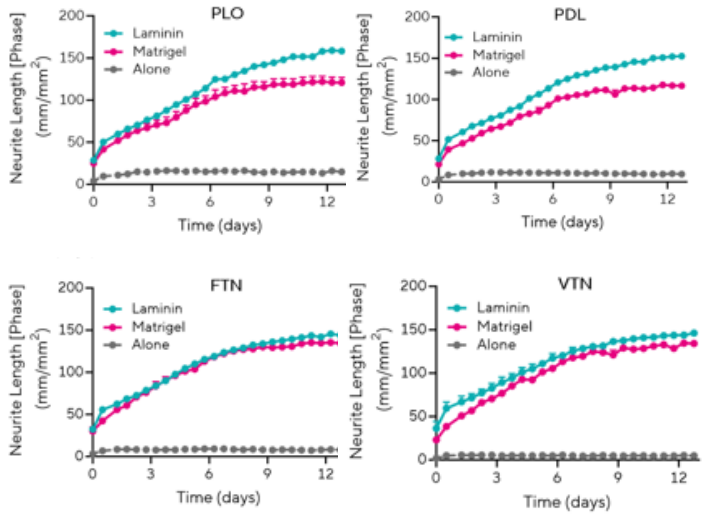

Figure 1. Improved neurite outgrowth using ABM's xeno-free coatings.

Strategy #2: Co-Culture

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

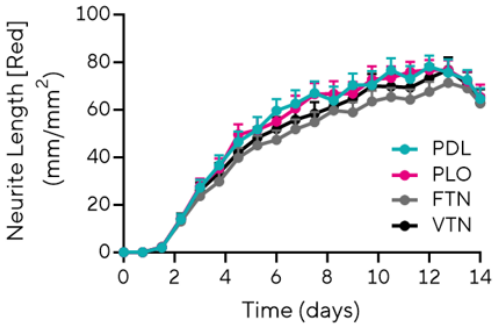

Figure 2. Increasing trend of neurite length with ABM's xeno-free coatings.

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Xeno-Free Dual Coatings Improve Neuronal Outgrowth Models

Abstract

This study aimed to evaluate the growth of human iPSC-derived neurons on Advanced Biomatrix's (ABM) xeno-free coatings, comparing dual and single coatings. Additionally, iPSC-derived neurons were co-cultured with iPSC-derived astrocytes to increase the physiological relevance and to test ABM's matrices. The results showed that dual coatings with ABM solutions effectively supported co-cultures of neurons and astrocytes and significantly improved neurite outgrowth.

Table 1. Tissue culture plate coating solutions for neuronal assays.

Materials	Supplier	Cat. No.	Final Concentration
Poly-D-Lysine	Advanced BioMatrix	5049	100 µg/mL
Poly-L-Ornithine	Advanced BioMatrix	5058	100 µg/mL
Fibronectin	Advanced BioMatrix	5050	50 µg/mL
Vitronectin	Advanced BioMatrix	5051	50 µg/mL
Laminin 511	Advanced BioMatrix	5344	3.3 µg/mL
Polyethylenimine	Sigma	408727	2.2 mg/mL
Matrigel®	Corning™	356231	28 µg/mL

Introduction

Induced pluripotent stem cells (iPSCs) are created by reprogramming adult cells into a stem-cell-like state, allowing them to develop into various cell types, including neurons.[2] Neurons have complex structures like dendrites and axons that enable precise communication between cells.[3] However, neurons are highly sensitive to environmental changes, making it difficult to culture and accurately study their properties. Hence, it is essential to culture iPSCs in conditions that closely mimic their inherent physiological environments. This is done by coating tissue culture plates with biologically relevant materials that can support neuron growth and activity. Xeno-free reagents are especially useful, as they remove foreign contaminants, improving experiment consistency and accuracy. This study demonstrates how ABM's xeno-free solutions can be used as coatings to promote successful neurite outgrowth in iPSC-derived neurons co-cultured with astrocytes.

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Results

Dual-coating of ABM's Xeno-free solutions improved neurite outgrowth for iPSC-derived neurons compared to single-coating

Phase images of iPSC-derived neurons on coated plates showed successful cell attachment (yellow) across all culture conditions. All base coatings supported neurite outgrowth, with more neurites (pink) seen on dual-coated plates compared to single-coated ones. (Figure 3A). Neurite length quantification over time confirmed longer neurites for dual-coated plates, comparable to the PEI control (Figure 3B). ABM's xeno-free solutions showed similar or better neurite outgrowth than Matrigel®, offering an alternative matrix for neuronal assays.

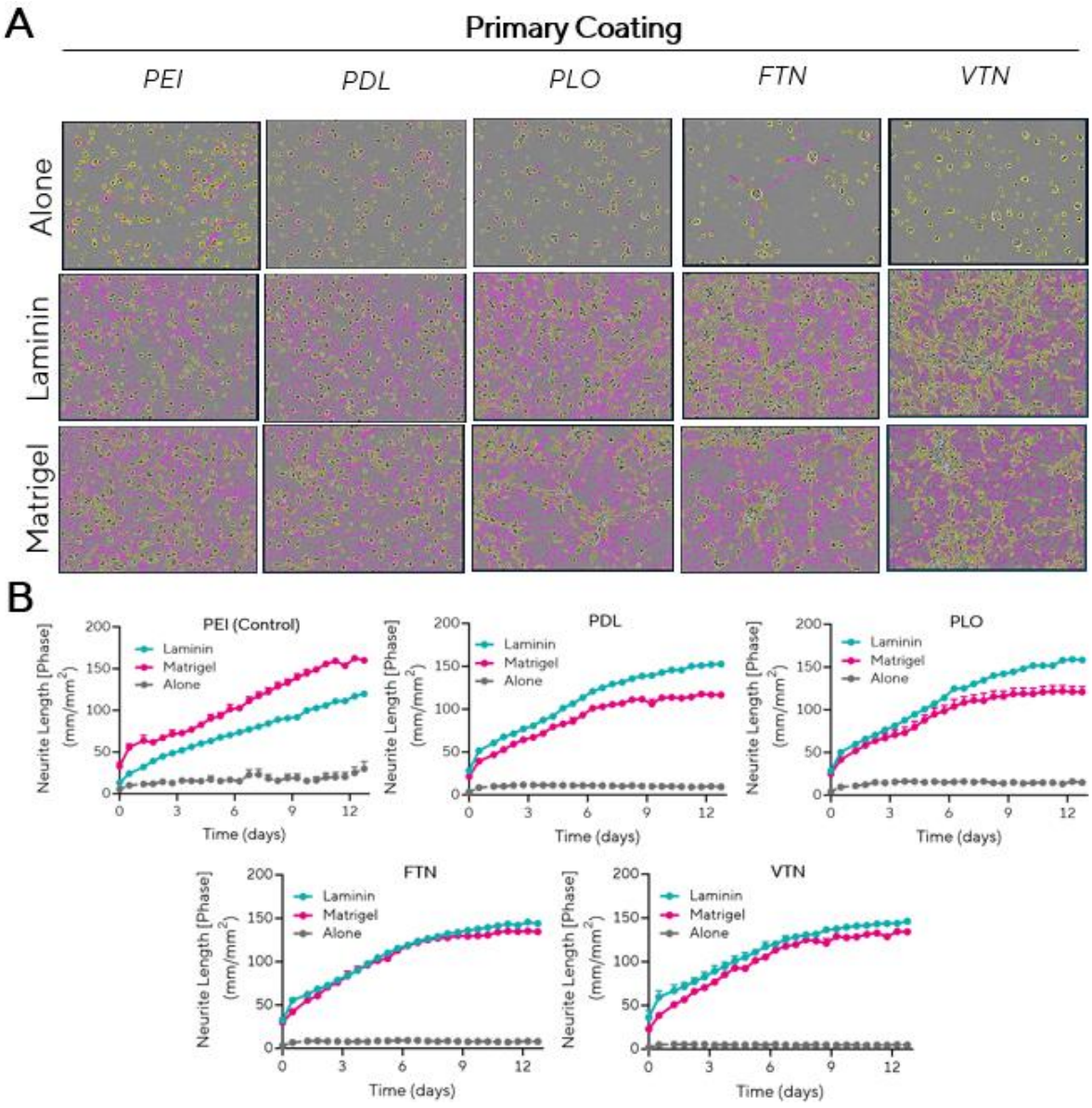

Figure 3. Neurite outgrowth of iPSC-derived neurons on ABM'S xeno-free coatings. (A) Masked cell bodies (yellow) and neurites (pink) shown at 12 days in vitro. (B) Quantified neurite length of cells cultured in single-coated and dual-coated plates (data represents mean ± SEM).

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ABM's Xeno-free coatings enable robust neurite outgrowth of human iPSC-derived neurons co-cultured with astrocytes

Representative phase and fluorescent images of the co-cultured astrocytes and neurons show robust neurite outgrowth for all ABM coating conditions at days in vitro (DIV) 14 (Figure 4A). Quantification of neurite length via fluorescence showed a robust increase in neurite outgrowth over time with little-to-no differences between base coatings (Figure 4B), demonstrating the ability of a variety of ABM solutions to support iPSC-derived co-cultures.

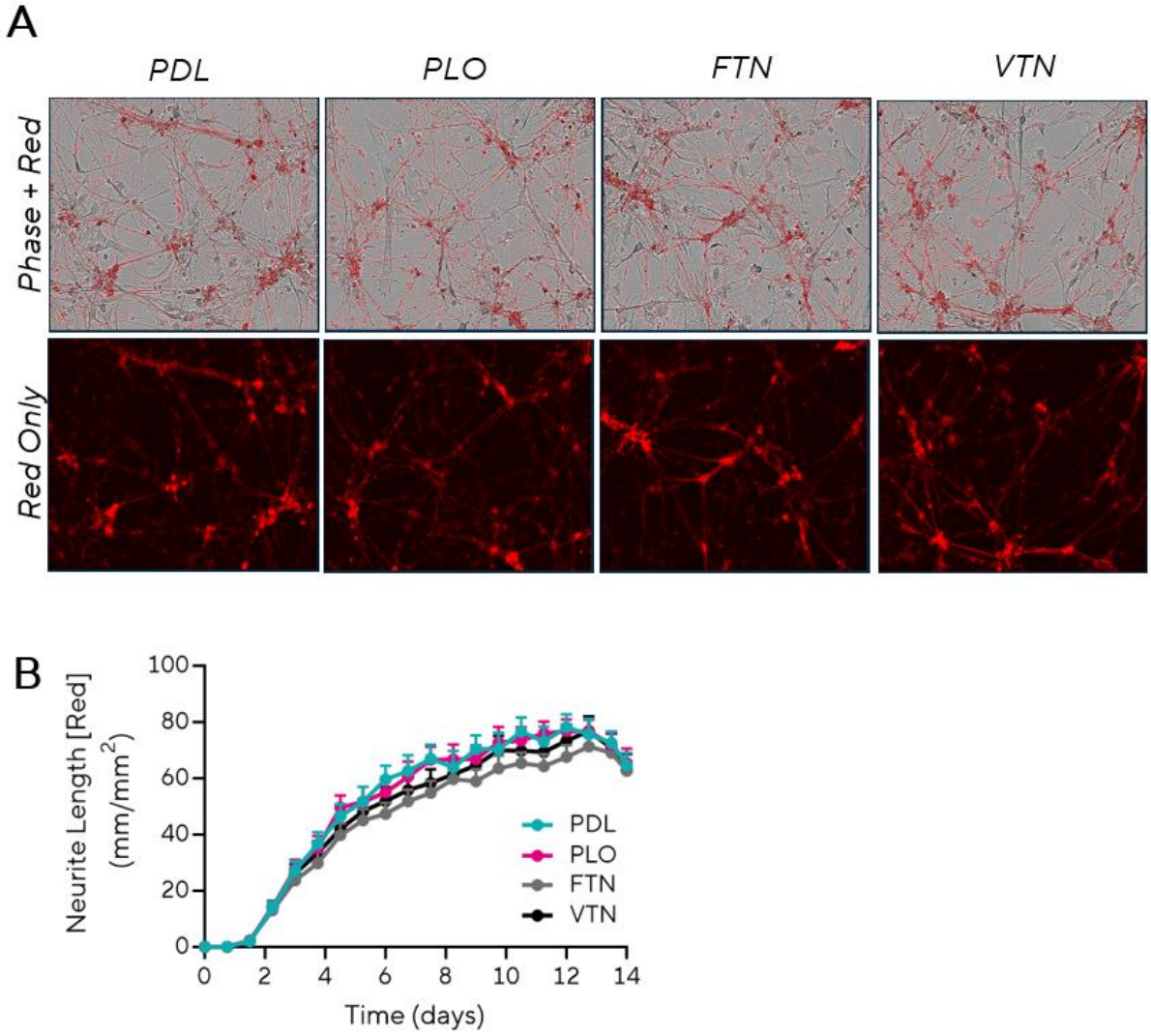

Figure 4. Neurite outgrowth of iPSC-derived neurons co-cultured with astrocytes on xeno-free coatings. (A) Representative phase and/or fluorescent images of neurite outgrowth. (B) Neurite length quantified over time and comparison across all coating conditions (data represents

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Conclusion

In summary, this study showed that dual coatings of ABM's xeno-free solutions improved neurite outgrowth compared to single coatings. In addition, ABM's xeno-free biomatrices successfully support the co-culture of iPSC-derived neurons and astrocytes, creating more accurate models for neuronal research. These co-cultures offer a clinically relevant model for studying neurological diseases, aiding future translational applications in human neurophysiology.

Methods and Materials

Cell Culture & Plate Coating

96-well plates were coated with ABM solutions at the indicated concentrations (Table 1). Xeno-free solutions were added (100 µL) to each 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 or Matrigel® was added on top of the base coating, 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

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Neurite Outgrowth Assay

As shown in Figure 5, 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 cell attachment. For co-cultures, Incucyte® Neurolight Orange Lentivirus in complete BrainPhys medium (100 µL/well) was added and incubated for 16-24 hours before being replaced with fresh media. iPSC-derived human astrocytes were then added at 15,000 cells/well. 48 hours after plating, 5-Fluoro-2'deoxyuridine and uridine (FdU/U) were added to stop astrocyte proliferation. Both monocultures and co-cultures were maintained in complete BrainPhys media (200 µL/well), with a 50% media change every other day. HD phase images were taken every 6 hours and analysed using the Incucyte® Neurotrack Analysis Software.

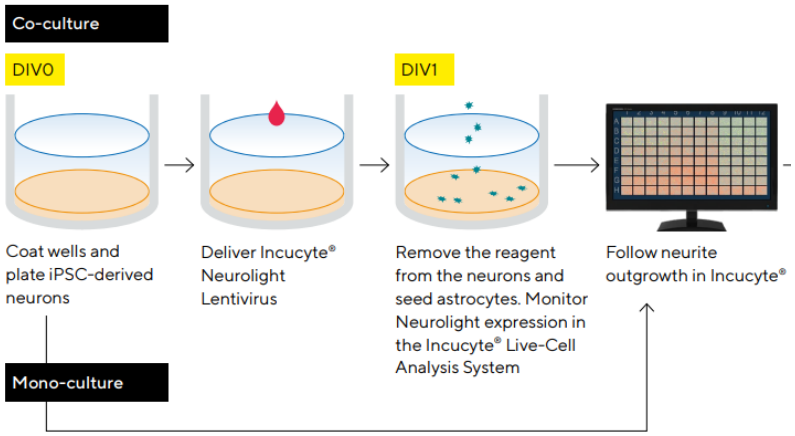

Figure 5. Workflow of neurite outgrowth assay.

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1. Harris E. Neurological Conditions Are Leading Cause of Disability Worldwide. JAMA. 2024;331(17):1440.
2. Yee J. Turning Somatic Cells into Pluripotent Stem Cells. Nature Education. 2010;3(9):25.
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