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Coating of Cell Culture Vessels for Mono-culture of hiPSC-Derived Motor Neurons

iXCells™ Human iPSC-Derived Motor Neurons can be cultured on different coating matrixes. The comparison among these coating conditions is listed in **Table 1**.

Table 1. Comparison of different types of coating for iPSC-motor neurons (iPSC-MNs).

Coating Matrix	Preparation Time	Cell Morphology	Applications
Matrigel or Cultrex	2 hours	• Low affinity to the plate • High mobility • Cell clusters will be observed after a few days in culture, usually connected by straight neurites and covered by cell debris • Suitable for short-term culture. Neurites will form bundles and the cells may detach from the plate after 7-10 days **(Figure 1)**	**Short-term culture (< 7 days)** • Gene Expression
Poly-D-Lysine (PDL) + Matrigel	> 24 hours	• Medium affinity to the plate • Medium mobility • Cell clusters will be observed after a few days in culture, usually connected by straight neurites and covered by cell debris • Suitable for long-term culture (>7 days), although neurites will form bundles. 50% medium change every 3-4 days gently to avoid cell detachment **(Figure 2)**	**Long-term culture (7-60 days or longer)** • Gene Expression • Immunofluorescence Staining
Polyethyleneimine (PEI) + Laminin	> 24 hours	• High affinity to the plate • Low mobility • No big cell clusters or straight neurites. The cell debris evenly distributed in the plate • Suitable for long-term culture (>7 days), no neurite bundles. 50% medium change every 3-4 days gently to avoid cell detachment **(Figure 3)**	**Long-term culture (7-60 days or longer)** • Gene Expression • Immunofluorescence Staining • Image-Based Assay • Neurite Outgrowth Assay • Electrophysiological Activity (MEA)

Matrigel Coating Preparation (96-well plate)

1. Dilute the Corning Matrigel GFR membrane matrix (Corning, Cat# 354230) or Cultrex RGF Basement Membrane Extract (R&D system, Cat#3433) to 80 µg/mL with DMEM/F12 (ThermoFisher Cat#11320082) medium.
2. Add 50 µL of the diluted Matrigel into each well of 96-well cell-treated plates to cover the surface. Coat the plates at room temperature for at least 2 hours or 37°C for at least 1 hour before use. The coated plates can be stored at 4°C for a week.

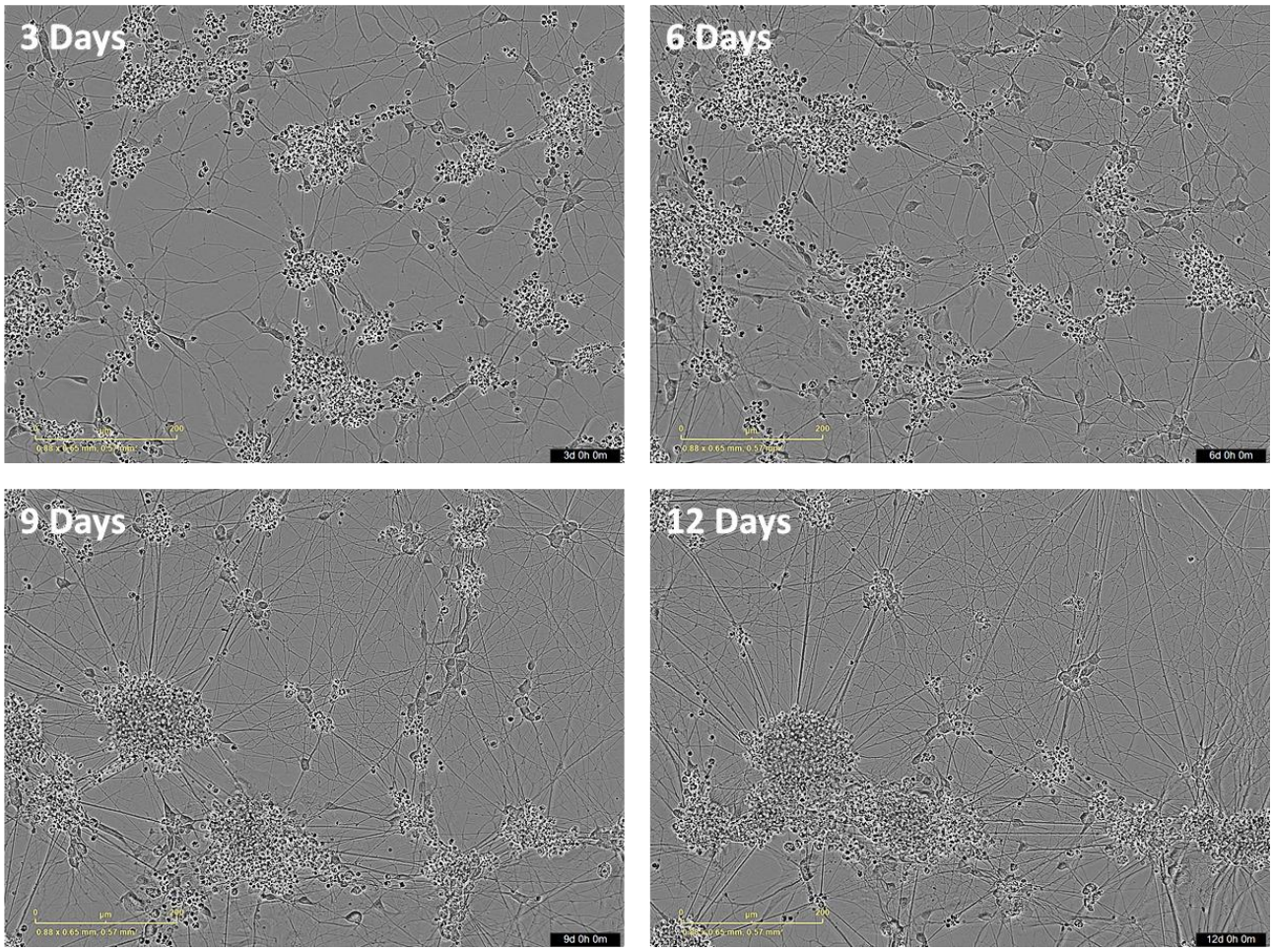

Figure 1. Motor neurons (Normal, Cat# 40HU-005) were seeded at the density of 50,000 viable cells per well in Motor Neuron Maintenance Medium (Cat# MD-0022) on Matrigel-coated 96-well cell-culture plates for 12 days. The cell morphology was monitored in real-time using Essen BioScience IncuCyte® S3. The phase contrast images are shown at 3 days, 6 days, 9 days, and 12 days post-thaw. Scale bar: 200 µm.

PDL + Matrigel Coating Preparation (96-well plate)

1. Dilute the Poly-D-Lysine (PDL) stock solution (1.0 mg/mL; Sigma-Aldrich, Cat# A-003-E) to 10 µg/mL with sterile water.
2. Dilute the Corning Matrigel GFR membrane matrix (Corning, Cat# 354230) to 80 µg/mL with DMEM/F12 (ThermoFisher, Cat#11320082) medium.
3. Transfer 50 µL of the diluted PDL solution into each desired well of a 96-well cell-culture treated plate. Incubate overnight inside a biosafety cabinet.
4. On the second day, aspirate the PDL solution from the overnight coated plate, and then wash twice with DPBS.
5. Add 50 µL of the diluted Matrigel solution into each well to cover the surface. Leave the plate undisturbed at room temperature for at least 2 hours or 1 hour in 37 °C CO_2 incubator before use. The coated plates can be stored with Matrigel at 4°C for a week. Aspirate Matrigel just before seeding the cells.

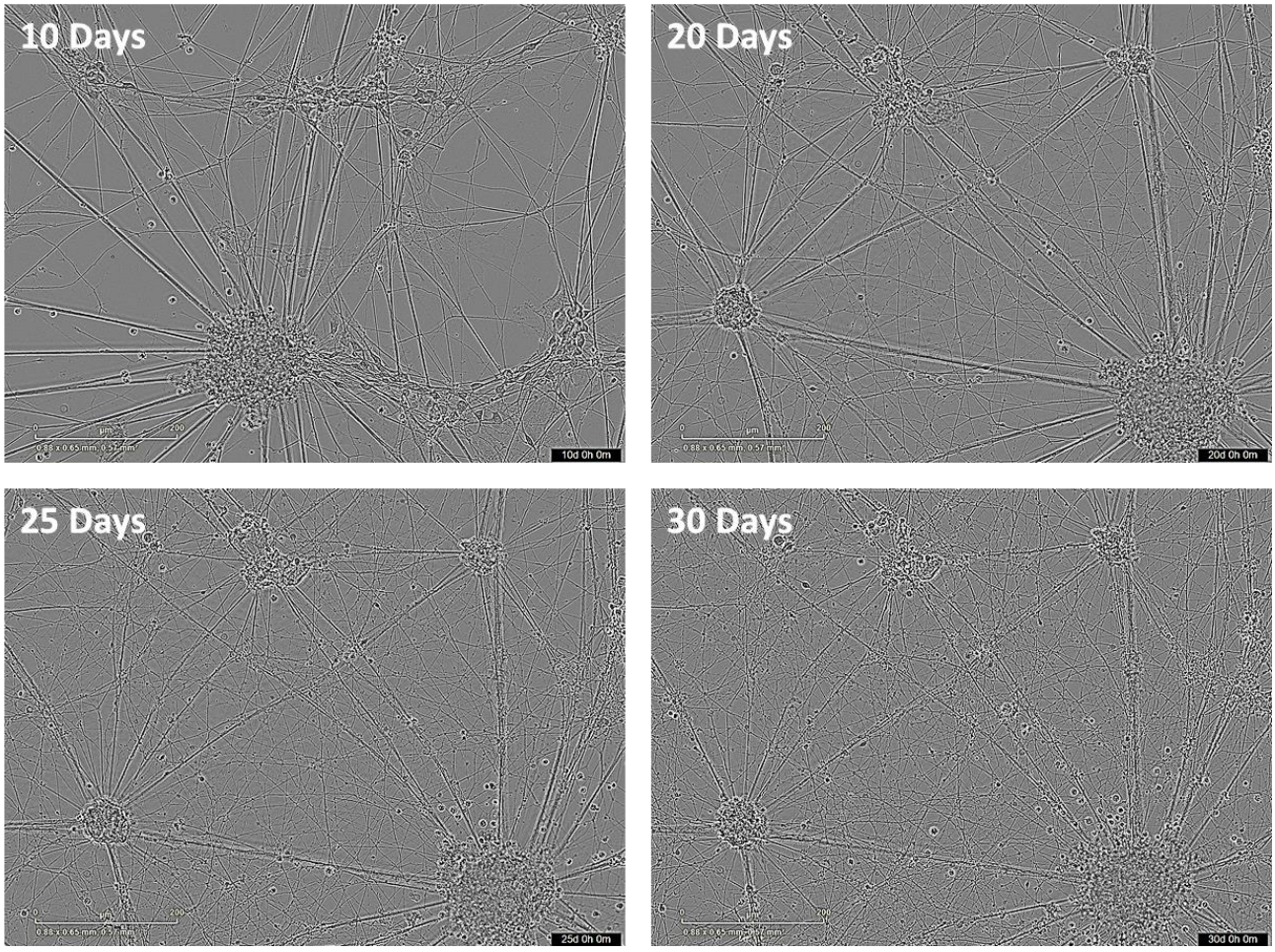

Figure 2. Motor neurons (Normal, Cat# 40HU-005) were seeded at the density of 30,000 viable cells per well in Motor Neuron Maintenance Medium (Cat# MD-0022) on PDL + Matrigel double coated 96-well cell culture plates for 30 days. The cell morphology was monitored in real-time using Essen BioScience IncuCyte® S3. The phase contrast images are shown at 10 days, 20 days, 25 days, and 30 days post-thaw. Scale bar: 200 µm.

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PEI + Laminin Coating Preparation (96-well plate)

1. Dilute the Polyethyleneimine (PEI) stock solution (Sigma-Aldrich, Cat#181978) to 7% with sterile water.
2. Dilute the 20X Borate Buffer (ThermoFisher, Cat#28341) to 1X Borate Buffer with sterile water.
3. Mix the 7% PEI with 1X Borate buffer to the desired volume (Add 7% PEI 1:100 to 1X borate buffer).
4. Transfer 50 µL of the mixed solution into each desired well of a 96-well cell-culture treated plate to cover the surface. Incubate the plate for 1 hour in 37°C CO_2 incubator.
5. Wash each coated well twice with 100 µL PBS, and then add 100 µL sterile water.
6. Aspirate the sterile water and leave the plate inside a biosafety cabinet overnight to dry out completely.

   Note: *Removing the lid while drying is recommended.*
7. On the second day, dilute Laminin stock solution (1mg/mL; Sigma-Aldrich, Cat#L2020) to 5 ug/mL with sterile water.
8. Add 50 µL of the working Laminin solution into each well to cover the surface. Leave the plate undisturbed at room temperature for at least 2 hours or 1 hour in 37°C CO_2 incubator before use. The coated plates can be stored with Laminin at 4°C for a week. Aspirate the Laminin solution just before seeding the cells.

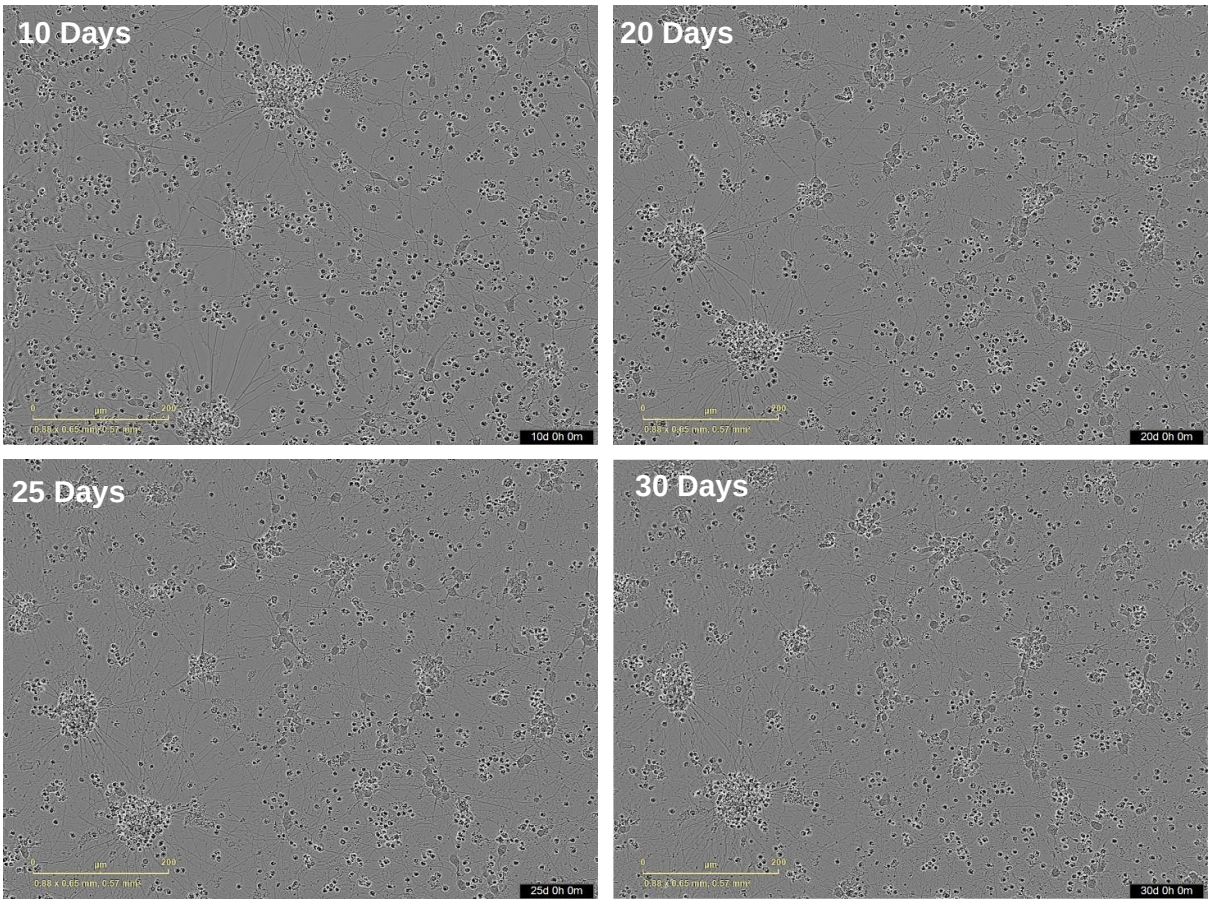

Figure 3. Motor neurons (ALS, Cat# 40HU-006) were seeded at the density of 30,000 viable cells per well in Motor Neuron Maintenance Medium (MD-0022) on PEI + Laminin double coated 96-well cell culture plates for 30 days. The cell morphology was monitored in real-time using Essen BioScience IncuCyte® S3. The phase contrast images are shown at 10 days, 20 days, 25 days, and 30 days post-thaw. Scale bar: 200 µm.

Disclaimers

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