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Corning® PureCoat™ Amine Supports Cell Attachment and Differentiation of Neural Cells

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Introduction

Cultured neural cells are used in vitro as a method to study brain function and metabolism. Interestingly, brain cells are fastidious to culture and are weakly adherent on standard culture vessels. These cells prefer vessels with coatings such as poly-d-lysine, poly-ornithine, and laminin. Many researchers self-coat vessels to enhance neural cell attachment, a time-consuming process which may result in lot-to-lot and plate-to-plate inconsistencies, often leading to experimental rework. Surface coatings have profound effects on neural cell morphology and function and influence the formation of neurite outgrowths and branches, a hallmark of neural cell differentiation. This application note reports that Corning PureCoat amine supports both neural cell attachment and differentiation.

Materials and Methods

Rat cerebellar granule cells

Rat cerebella from 6-12 days old pups were purchased from Charles River Laboratories (MA) and cells were freshly isolated based on previously published protocols[1]. Briefly, tissues were crushed with a cell scraper and digested with trypsin and DNAse solution. The tissue mixture was repeatedly aspirated and ejected using a 25 ml pipet yielding a cell suspension. Resulting solutions were then mixed with DNAse and Soybean trypsin inhibitor to stop the trypsinization process. The resulting single cell suspension was then centrifuged and supernatant discarded. The cell pellet was resuspended in growth medium (Eagle's medium containing 10% FBS, L-Glutamine, 0.001% Potassium Chloride, and Gentamycin). An aliquot was removed for cell counting. Rat cerebellar granule cells were seeded at ~450,000 cells/cm^2 on 24-well tissue culture-treated (TC) and Corning PureCoat amine plates in 0.5 ml/well of growth medium for 24- or 48-hours in a humidified 37°C incubator.

Rat cortical neuronal cells

A vial of cryopreserved primary rat brain cortex neuronal cells were purchased from Lonza (Cat. No. R-Cx-500), thawed and resuspended in 10 ml of growth medium (Lonza, Cat. No. CC-4461) according to the manufacturer's recommendations. Then, a 1 ml/well volume of cell suspension was seeded onto 24-well tissue culture-treated and Corning PureCoat amine plates. Care was taken to minimize any changes in osmolality as these cells are very sensitive to such changes. Medium was replenished the next day and cells were allowed to grow. On the fifth day, spent medium was aspirated and refreshed with fresh growth medium. Images were then captured using a 20X objective on an Olympus microscope.

Cell attachment assay

The CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (MTS) (Promega™, Cat. No. G3581) was utilized for attachment and growth studies. MTS is a tetrazolium compound that is reduced by metabolically active living cells into a soluble product, formazan, which gives a purple hue. The amount of formazan product is directly proportional to the number of living cells. Therefore, cell proliferation or attachment can be quantified by reading the absorbance of formazan. MTS was added directly into culture medium and plates were then read on a Tecan® Safire2™ microplate reader at 490 nm. A row of wells containing media plus MTS served as experimental blank and data were expressed as background-subtracted values.

Rat astrocytes

Cryopreserved primary rat astrocytes were purchased from Lonza (Cat. No. R-CxAS-520), thawed and resuspended in Astrocyte growth medium (Lonza, Cat. No. CC-3186) according to the manufacturer's recommendations. Cells were then seeded at ~10,000 cells/cm^2 on 96-well tissue culture-treated, enhanced TC Corning CellBIND® multiple well plates, and Corning PureCoat amine plates in a 200 µl/well volume of growth medium. The next day, medium was replenished and cells were allowed to grow. On the fifth day, spent medium was aspirated and medium containing MTS (Promega, Cat. No. G3581) was added to quantify cell attachment/growth.

Immunocytochemistry

Cell cultures were washed with 1X Phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 10 minutes at 4°C. Next, cells were washed with 1X PBS, blocked with 3% fetal bovine serum and incubated with 1:10 dilution of the primary mouse anti-β-tubulin directly conjugated to Alexa Fluor® 488 (BD Cat. No. 558605) for 1.5 hour at room temperature. Cultures were then washed with 1X PBS and stained with a 1:1000 dilution of a 1 µg/ml DAPI solution, a nuclear dye. No-antibody controls for staining were also run in parallel. Immunostained cells were visualized with a 10X objective on a BD Pathway™ 800 Imager. Montage settings were utilized to image larger areas of cells.

Staining with Calcein AM fluorescent dye

Rat astrocyte cultures were washed with serum-free, phenol red-free DMEM and incubated with 5 µg/ml of Calcein AM fluorescent dye (Corning, Cat. No. 354216) in the same medium at 37°C for 1-hour. Images were then captured with a 10X objective on a fluorescence microscope.

Results and Discussions

There exists a breadth of information supporting the requirement of specialized surfaces for the attachment of neural cells. It also appears that positively charged surfaces are more popular for neural cell attachment. We report that Corning® PureCoat™ amine, a positively charged surface, supports attachment and differentiation of a variety of primary neural cell types including rat cerebellar granule, rat astrocytes, and rat cortical neuronal cells. This improvement in attachment was observed both with freshly isolated as well as cryopreserved neural cells post-thaw. Rat cerebellar granule cells, isolated from rat brains, attached on Corning PureCoat amine and neurite outgrowths were observed within 24-hours. Cells on Corning PureCoat amine surface continue to express typical phenotypic markers of neuronal cells such as tubulin. It appeared that neurite outgrowths traveled longer distances by 48-hours (**Figure 1A-1C**).

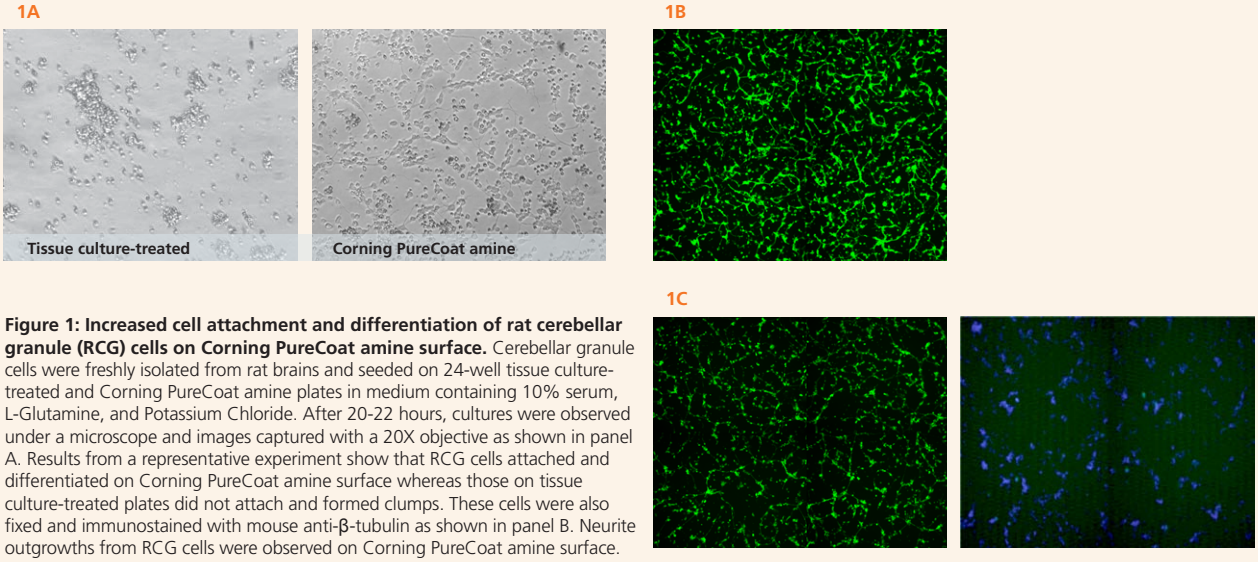

Figure 1: Increased cell attachment and differentiation of rat cerebellar granule (RCG) cells on Corning PureCoat amine surface. Cerebellar granule cells were freshly isolated from rat brains and seeded on 24-well tissue culture-treated and Corning PureCoat amine plates in medium containing 10% serum, L-Glutamine, and Potassium Chloride. After 20-22 hours, cultures were observed under a microscope and images captured with a 20X objective as shown in panel A. Results from a representative experiment show that RCG cells attached and differentiated on Corning PureCoat amine surface whereas those on tissue culture-treated plates did not attach and formed clumps. These cells were also fixed and immunostained with mouse anti-β-tubulin as shown in panel B. Neurite outgrowths from RCG cells were observed on Corning PureCoat amine surface. In some instances, cultures were incubated for up to 48-hours, and stained with anti-β-tubulin and a nuclear dye, DAPI as shown in panel C. Rat cerebellar granule cells grown on Corning PureCoat amine exhibit several dendritic processes and express tubulin, a neuronal marker. RCG cells cultured on tissue culture-treated plates remained unattached and most were washed away during the fixing protocol (data not shown).

A similar observation was made with rat brain cortex-derived neurons, when cryopreserved neuronal cells were plated immediately post-thaw onto Corning PureCoat amine surface. Cells attached and differentiated, and a network of neurite outgrowths were observed on Corning PureCoat amine surface within five days. However, cells seeded on tissue culture-treated surface remain unattached and formed large floating clumps (**Figure 2A and 2B**).

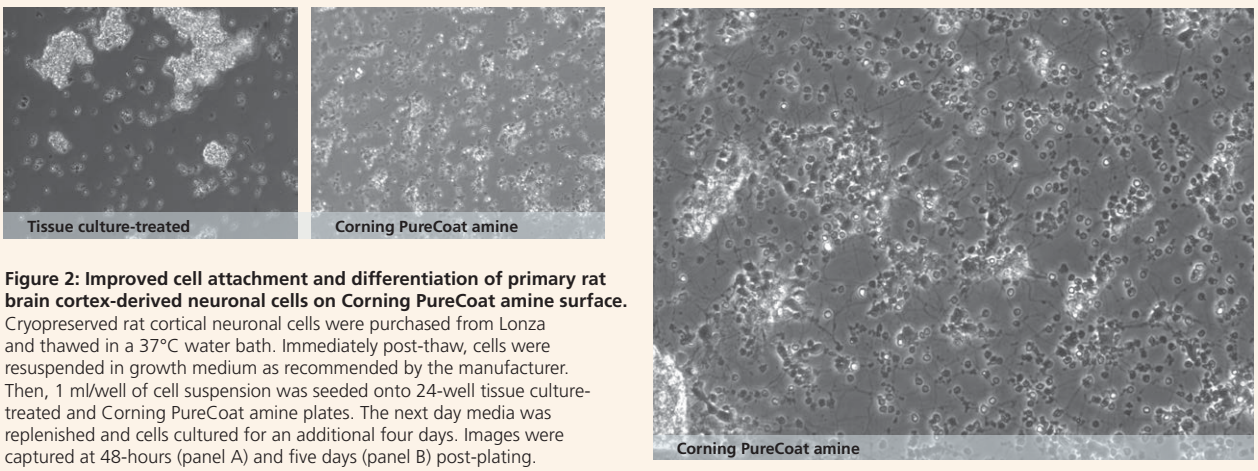

Figure 2: Improved cell attachment and differentiation of primary rat brain cortex-derived neuronal cells on Corning PureCoat amine surface. Cryopreserved rat cortical neuronal cells were purchased from Lonza and thawed in a 37°C water bath. Immediately post-thaw, cells were resuspended in growth medium as recommended by the manufacturer. Then, 1 ml/well of cell suspension was seeded onto 24-well tissue culture-treated and Corning PureCoat amine plates. The next day media was replenished and cells cultured for an additional four days. Images were captured at 48-hours (panel A) and five days (panel B) post-plating. Cells on tissue culture vessels did not attach and formed clumps which increased in size over time. Cells seeded onto Corning PureCoat amine plates remained attached as shown in panel A. Neurite outgrowths from cortical neuronal cells formed a network within five days as shown in panel B, whereas those on tissue culture remain clumped and unattached (data not shown). Neurites were observed within 48-hours post-plating but continued to increase over time.

In both instances, the majority of the attached cells were spherical in shape and within 24-hours cells possessed small processes which multiplied and increased in size. The formation of these neurites is dependent on the number of seeded cells. It was also found that low cell density seeding did not support good neurite growth.

Astrocytes are the support cell type of the neural system and are often found in close association with neurons. We demonstrated that Corning® PureCoat™ amine supports the attachment of primary rat astrocytes post-thaw. Cell growth on Corning PureCoat amine was also 50% greater over a six-day period than on tissue culture-treated or enhanced TC Corning CellBIND® vessels (**Figure 3A**). Images of rat astrocytes stained with Calcein AM fluorescent dye show more cells on Corning PureCoat amine and concur with the growth assay (**Figure 3B**).

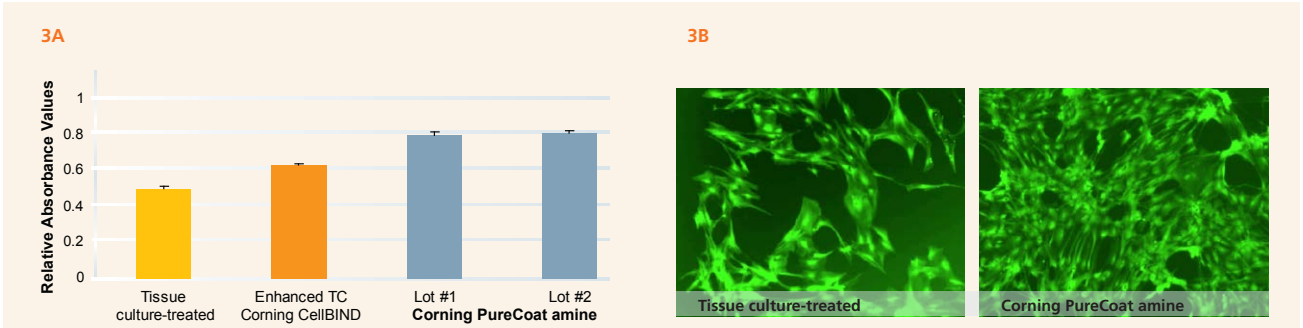

Figure 3: Better cell attachment and growth of primary rat astrocytes on Corning PureCoat amine surface. Cryopreserved cells purchased from Lonza were thawed in a 37°C water bath and immediately seeded onto 96-well tissue culture-treated, enhanced TC Corning CellBIND multiple well plates, and Corning PureCoat amine plates at ~10,000 cells/cm^2 in 200 µl of growth media recommended by the manufacturer. Media was replenished 24-hours post-plating and on day six, spent medium was aspirated and subjected to MTS quantification. Enhanced cell growth of primary rat astrocytes cultured on Corning PureCoat amine (>50% vs. tissue culture) surface post-thaw was observed as shown in panel A. In some instances, cells were also stained with 5 µg/ml of Calcein AM fluorescent dye for one hour at 37°C in serum-free DMEM for better visualization. Images were captured with a 10X objective on a fluorescence microscope as shown in panel B. More astrocyte cells attached on Corning PureCoat amine surface than on tissue culture vessels.

Conclusions

- Corning PureCoat amine surface supports the attachment and growth of neural cell types that include neuronal and glial cells.
- Corning PureCoat amine surface supports differentiation of primary neuronal cells.

Acknowledgments

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Reference

1. Swiderek, M.S., Mannuzza, F.J., Ilsley, S.R., and Myles, A. *Preparation of a Cell Culture Substrate Coated with Poly-D-Lysine*. (1997).

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