Optimizing neuron adhesion and growth by choosing the right surface for the Nunc Chamber Slide

Key words

Lab-Tek II CC^2 Chamber Slide, primary neuron, PC12, primary amines.

Introduction

Thermo Scientific™ Nunc™ Lab-Tek™ II CC$^{2™}$ Chamber Slides are designed to simplify imaging analysis involving microscopic examination of cultured cells. The multi-chambered design allows for parallel studies of multiple conditions and stimuli required by many applications. The downstream cyto-staining process is greatly facilitated by the removal of the upper structures (wells). The standard footprint of a slide is compatible with all imaging equipment, making microscopic examination very convenient.

In general, chamber slides are used for cellular imaging studies of adherent cells. Unlike many anchorage-dependent cell types, neurons do not adhere easily to standard cell culture–treated surfaces. Neuron survival and growth depend heavily on certain biological coatings or chemical modifications of the culture surface. In this study, we examined the correlation between neuronal cell adhesion and the chamber slide surface modifications.

Methods

Cell culture

Rat pheochromocytoma, PC12 (ATCC™ CRL-1721™), cells were maintained in Gibco™ MEM containing L-glutamine (Cat. No. 11095072), Antibiotic-Antimycotic solution (Cat. No. 15240062), Non-Essential Amino Acids (Cat. No. 11140050), Sodium Pyruvate (Cat. No. 11360070), 10% horse serum (Cat. No. 16050130), and 5% bovine calf serum (Cat. No. 16170060). Three days after plating on test surfaces, the medium was replaced with an N2.1 defined medium (modification of [2] and [3]) containing progesterone (20 nM), putrescine (100 nM), selenium dioxide (30 nM), 100 g/mL transferrin (bovine), 5 g/mL insulin (solubilized in 0.01 N HCl), and 0.5 mg/mL ovalbumin in Gibco™ DMEM (Cat. No. 11965084) with 15 mM HEPES (pH 7.36). Invitrogen™ nerve growth factor (NGF; Cat. No. 13257019, 100 ng/mL) was added after 4 days in culture. Cells were fixed 2 days after the addition of NGF. All cells were passaged on a weekly basis.

Primary chick brain cultures were prepared from 11-day-old chick embryos. Cortices were dissected, minced, incubated with trypsin for 20 minutes, carefully washed, and dissociated by drawing through a pipette. Cells were counted and plated at 10^5 cells/cm^2 in DMEM supplemented with 10% horse serum. The next day, the medium was changed to N2.1 defined medium. Cells were treated with a mitotic inhibitor on day 4. After 10 days in culture, the neurons were fixed in 4% formaldehyde and mounted with 90% glycerol or a permanent mounting buffer.

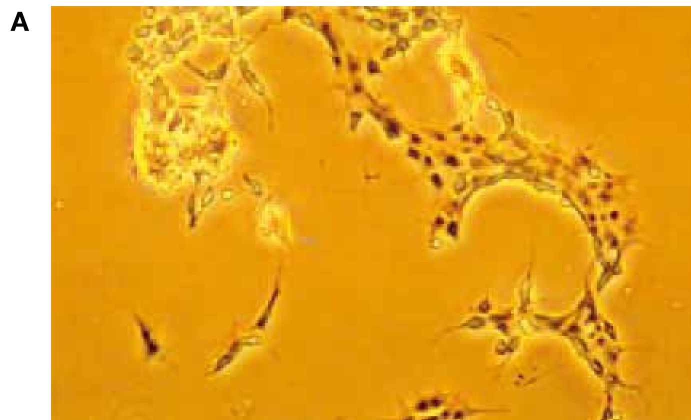
Modification of glass

Slides were dipped in 1% *N*-(2-aminoethyl)-3-aminopropyl-trimethoxysilane in 95% ethanol:water, washed two times with ethanol, and baked for 10 minutes at 100°C. This generated a surface with attached diaminopropyl silane (DAPS) groups. The procedure for the preparation of the Lab-Tek II CC² surface is proprietary information. All slides were assembled into chamber slide products and sterilized before use in cell culture. Chamber slide products requiring polylysine treatment were coated by incubating for 4–24 hours with filter-sterilized poly-D-lysine (PDL; 1 mg/mL) in borate buffer (boric acid 3.1 g/L and borax 4.8 g/L in water). They were washed with sterile water and dried before use.

Quantification of primary amines

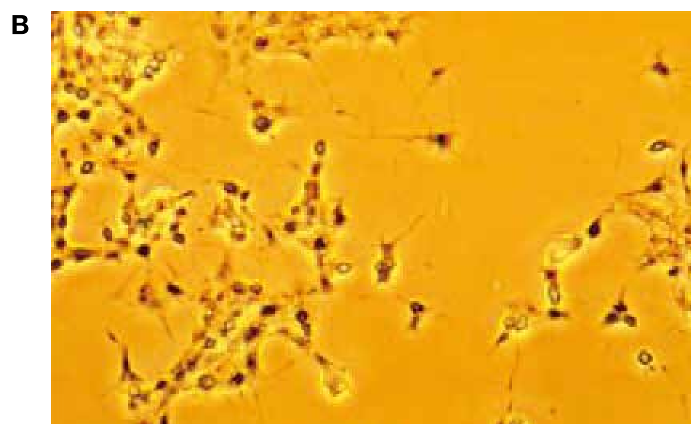
Primary amines were quantified using a Thermo Scientifc™ *o*-phthalaldehyde–based assay (Cat. No. 26025). All measurements were performed in soda lime glass chamber slide products or in chambered–cover glass assemblies using a fluorescence plate reader. A fluorescence vs. concentration curve was generated using PDL as the standard. The concentration of amines on the modified surfaces are expressed in relative terms of gram PDL/slide.

A



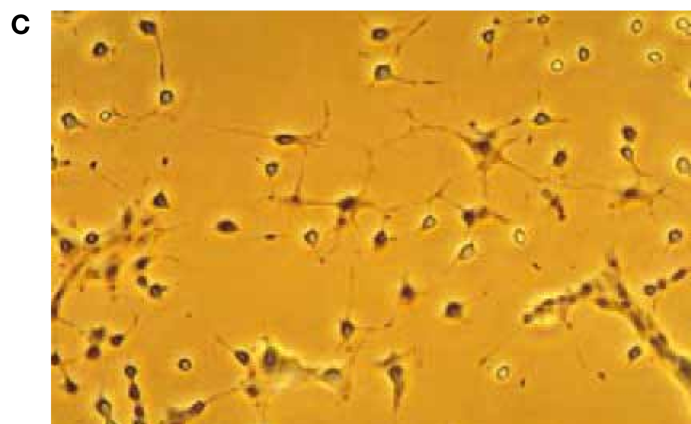
Unmodified

B



DAPS glass

C



Lab-Tek II CC² glass

Figure 1. PC12 cells displayed superior morphology and growth on the Lab-Tek II CC2–modified glass chamber slide compared to those on the unmodified glass and the DAPS glass.

Results

The cell line PC12 exhibits different behavior on modified and unnmodified glass

PC12 cells cultured on unmodified glass slides form cell clumps with fibroblast-like morphology. Cell death occurred in a significant portion of the culture, indicating suboptimal growth conditions for these cells on the unmodified glass (Figure 1A). PC12 cells appear less aggregated when grown on DAPS-modified glass; however, a large number of the cells still developed fibroblast-like morphology (Figure 1B). PC12 cells cultured on Lab-Tek II CC² glass demonstrated good survival. They are also less fibroblast-like with rounder bodies. The neuronal process outgrowth is more pronounced on Lab-Tek II CC² glass (Figure 1C) than that on the unmodified glass and the DAPS glass (Figures 1A, B).

Glass and plastic surfaces must be adequately modified to support adhesion and differentiation of primary neurons

Culturing primary neurons is particularly challenging since they do not continue to proliferate after dissociation. Primary neuron survival in culture depends on cell adhesion and differentiation, which can be facilitated by altering the culture surface with a biological coating or a chemical modification (Figure 2). Neuron adhesion and growth requires more than just the hydrophilic surface provided by the glass and standard cell culture–treated plastic surfaces. Therefore, very few neurons survived on bare glass or cell culture–treated Thermo Scientific™ Permanox™ slides (Figures 2A–C). The application of fresh PDL to the culture surface provides adequate surface chemistry for growth of primary neurons on both PDL-coated glass and PDL-coated Permanox chamber slides (Figures 2E–G), comparable to that of the PDL-coated polystyrene culture dish (Figure 2H). On the other hand, even without the PDL coating, Lab-Tek II CC^2 chemically modified glass provides a superior surface for adhesion and differentiation of primary neurons (Figure 2D).

Primary amines on the surface of coated and modified glass facilitate attachment and survival of neurons

Previous studies showed that neurons grown on surfaces modified with diamines and triamines had good neuronal cell morphology [3–9]. Similar observations were made with PDL-coated glass (glass slide and cover glass) and plastics (Permonox slide). Interestingly, by enriching the primary amines in the surface treatment, chemically modified Lab-Tek II CC^2 glass also supported neuronal cell growth, outperforming unmodified glass (Figure 2D) and the DAPS glass for primary neuron culture (data not shown).

Quantification of the primary amines present on the biologically coated surface and the chemically modified surface suggests a strong positive correlation between neuronal cell survival and the presence of amine functional groups on the culture surface (Table 1).

Table 1. Quantification of primary amines on glass surfaces

Culture surface	PDL equivalent (µg/slide)
PDL-coated glass	78
Lab-Tek II CC^2 glass	30
DAPS glass	5
Unmodified glass	<1

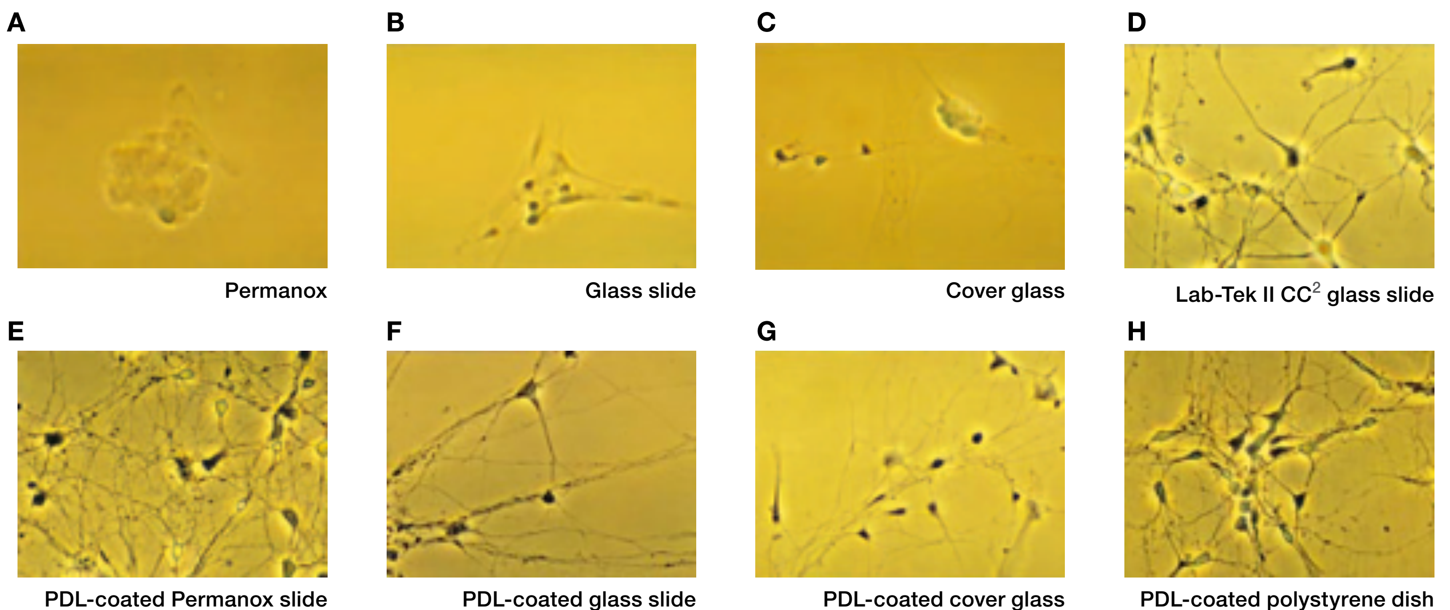


Figure 2. The growth and survival of primary neurons requires either a biological coating (e.g., polylysine) or a chemical modification (e.g., Lab-Tek II CC2 glass) of the chamber slide surface. Standard cell culture–treated plastics and bare glass fail to support primary neuron culture.

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Conclusions

While many cells prefer culture surfaces with high surface energies (i.e., hydrophilic surfaces), neuronal cells require the additional presence of primary amine groups on the growth surface. Chemically modified Lab-Tek II CC^2 Chamber Slide surfaces mimic the function of a PDL coating by providing sufficient surface chemistry (anime groups) to facilitate adhesion and differentiation of primary neurons.

Unlike the polylysine coating, the chemical modification of Lab-Tek II CC^2 slides is stable for more than 2 years at room temperature and requires no further coating before use.

References

1. Bottenstein JE, Sato GH (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc Natl Aca Sci USA* 76(1):514–519.
2. Bartlett W.P., & Banker G.A. (1984) An electron microscopic study of the development of axons and dendrites by hippocampal neurons in culture: 1. Cells which develop without intercellular contacts. *J. Neurosci* 4(8):1944–1953.
3. Corey JM, Wheeler BC, Brewer GJ (1991) Compliance of hippocampal neurons to patterned substrate networks. *J Neurosci Res* 30(2):300–307.
4. Healy KE, Thomas CH, Rezania A et al. (1996) Kinetics of bone cell organization and mineralization on materials with patterned surface chemistry. *Biomaterials* 17(2):195–208.
5. Kleinfeld D, Kahler KH, Hockberger PE (1988) Controlled Outgrowth of Dissociated Neurons on Patterned Substrates. *J of Neurosci* 8 (11):4098–4120.
6. Lom B, Healy KE, Hockberger PE et al. (1993) A versatile technique for patterning biomolecules onto glass coverslips. *J Neurosci Methods* 50(3):385–397.
7. Matsuzawa M, Potember RS, Stenger DA et al. (1993) Containment and growth of neuroblastoma cells on chemically patterned substrates. *J Neurosci Methods* 50(2):253–260.
8. Schaffner AE, Barker JL, Stenger DA et al. (1995) Investigation of the factors necessary for growth of hippocampal neurons in a defined system. *J Neurosci Methods* 62(1–2):111–119.
9. Spargo BJ, Testoff MA, Nielsen TG et al. (1994) Spatially controlled adhesion, spreading and differentiation of endothelial cells on self-assembled molecular monolayers. *Proc Natl Acad Sci USA* 91(23):11070–11074.

Ordering information

No. of wells	Suggested working volume (mL)	Culture area (cm^2/well)	Units per pack/carton/case	Cat. No.
Nunc Lab-Tek II Chamber Slide System, CC^2 glass				
1	2.0–4.5	8.6	8/16/96	154739
2	1.0–2.0	4.0	8/16/96	154852
4	0.5–1.0	1.7	8/16/96	154917
8	0.2–0.5	0.7	8/16/96	154941

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