Optimizing neuron adhesion and growth by choosing the right surface of Thermo Scientific Chamber Slide

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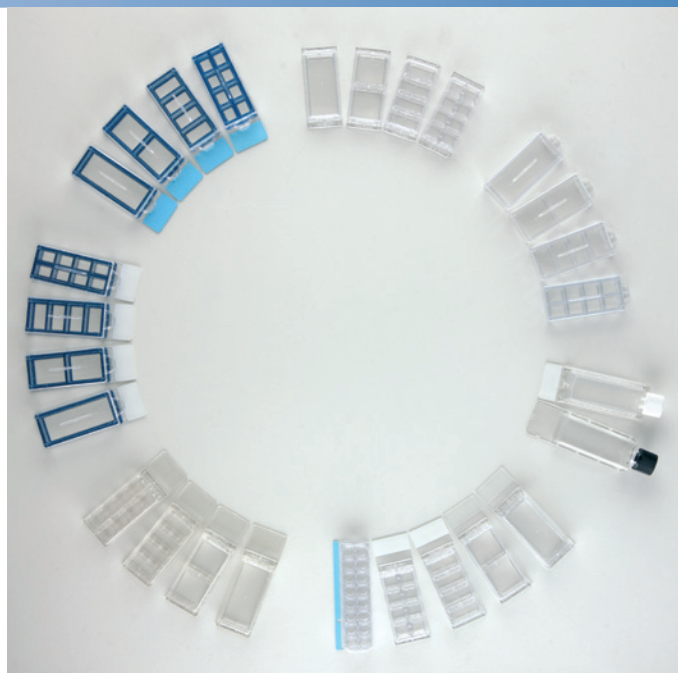
Key Words

Lab TeKII CC², Chamber slide, Primary Neuron, PC12, Primary Amines.

Goal

The goal of this application note is to showcase the advantage of specialty treated Thermo Scientific chamber slide glass for neuronal cell culture.



Introduction

Thermo Scientific Chamber Slides are designed for simplifying 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. Downstream cytostaining process is greatly facilitated by the removal of the upper structures (wells). The standard footprint of a slide is compatible with all imaging equipment and makes the microscopic examination very convenient.

In general, chamber slides are used for cellular imaging studies of adherent cells. Their ability to attach to various culture surfaces is cell type specific. For example, epithelial and fibroblast cell lines attach and proliferate readily on cell culture-treated plastic surfaces. Biological coatings and chemical modifications of the culture surfaces may alter the proliferation status of these cells. On the other hand, neurons do not adhere easily on standard cell culture-treated plastic surfaces. Their 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.

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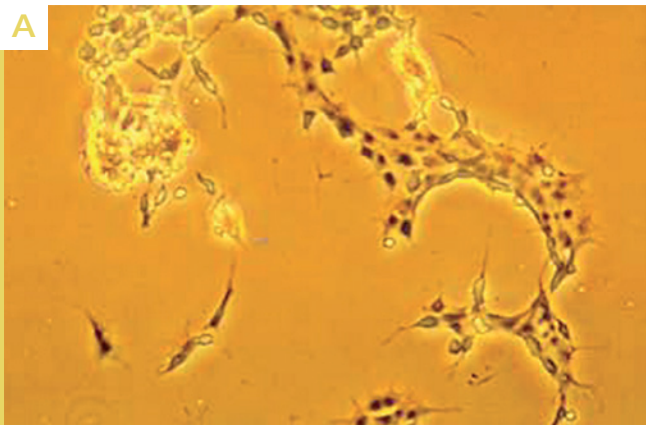
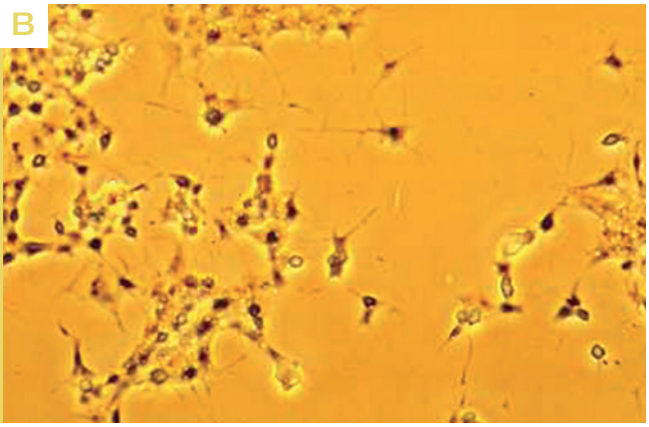
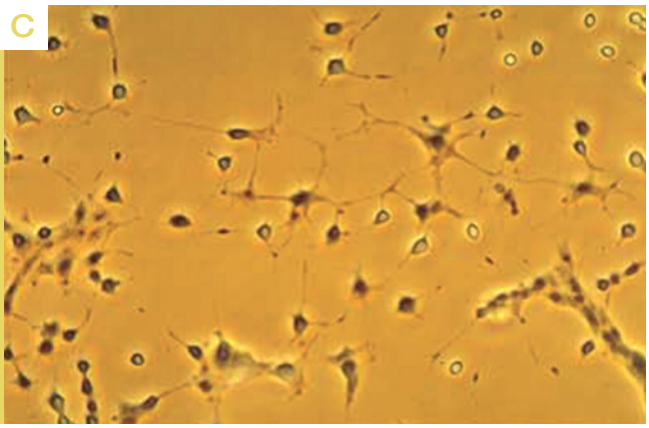


Non-modified



DAPS glass



CC^2 glass

Figure 1
PC12 cells displayed superior morphology and growth on the CC^2 modified glass chamber slide to those on the non-modified glass and the DAPS glass.

Methods

Cell Culture

PC12 (ATCC CRL 1721) cells were maintained in Thermo Scientific HyClone medium and supplements including MEM containing L-glutamine, antibiotic antimycotic, non-essential amino acids, sodium pyruvate, 10% horse serum and 5% bovine calf serum. Three days after plating on test surfaces, the medium was replaced with a N2.1 defined medium (modification of Bottenstein and Sato, 1979; Bartlett and Banker, 1984) containing progesterone (20 nM), putrescine (100), selenium dioxide (30 nM), 100 g/ml transferring (bovine), 5 g/ml insulin (solubilized in 0.01N HCI), and 0.5 mg/ml ovalbumin in DMEM with 15 mM HEPES (pH 7.36). Nerve growth factor (NGF; 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 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 105 cells/ cm2 in 10% horse serum/ DMEM (Thermo Scientific HyClone). The next day the medium was changed to N2.1 defined medium and on day 4, cells were treated with a mitotic inhibitor. After 10 days in culture the neurons were fixed in 4% formaldehyde and mounted in 90% glycerol or a permanent mounting buffer.

Modification of glass

Slides were dipped in 1% N-(2-aminoethyl)-3-aminopropyl trimethoxy silane 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 CC^2 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 to 24 hours with filter sterilized poly-D-lysine (PDL; 1 mg/ml) in borate buffer (boric acid 3.1 g/l, 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 an o-phthalaldehyde based assay (Thermo Scientific Pierce). All measurements were done in soda lime glass chamber slide products or in chambered-coverglass assemblies using a fluorescence plate reader. A fluorescence/ concentration curve was generated using polylysine as the standard. The concentrations of amines on the modified surfaces are expressed in relative terms of g polylysine/ slide.

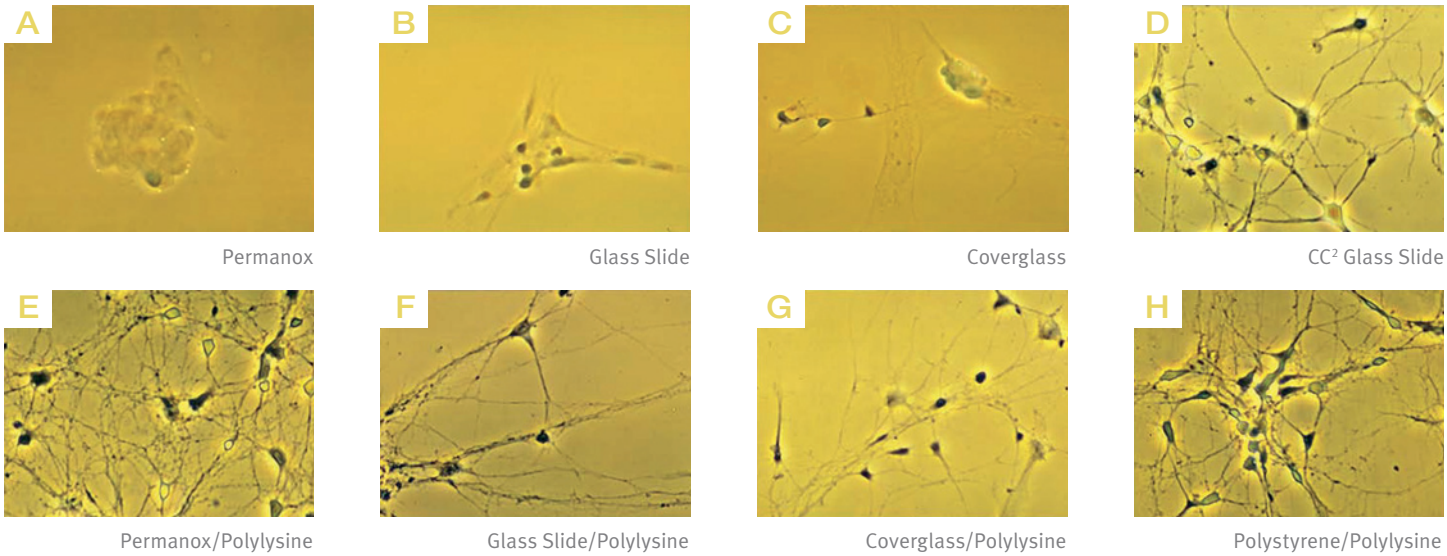


Figure 2
The growth and survival of primary neuron requires biological coating (e.g. polylysine) or chemical modification (e.g. CC² glass) of the chamber slide surface. Standard cell culture-treated plastics and bare glass fail to support primary neuron culture.

Results

The rat pheochromocytoma cell line PC12 shows differential behavior on modified and non-modified glass. PC12 cells cultured on non-modified glass slide form cell clumps with fibroblast-like morphology. Cell death occurred in significant portion of the culture indicating sub-optimal growth conditions for these cells on the non-modified glass (Figure 1A). PC12 cells appear less aggregated when grown on DAPS modified glass, although large number of the cells still developed fibroblast-like morphology (Figure 1B). PC12 cells cultured on CC² glass demonstrate good survival. They are less fibroblast-like with rounder bodies. The neuronal process outgrowth is more pronounced on CC² glass than that on the non-modified glass and the DAPS glass (Figure 1C).

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 proliferating after dissociation. Primary neuron survival in culture depends on cell adhesion and differentiation, which can be facilitated by altering the culture surface with biological coating or chemical modification (Figure 2). Neuron adhesion and growth requires more than just the hydrophilic surface provided by glass and standard cell culture-treated plastic surface. Therefore, very few neurons survived on bare glass or cell culture treated Permanox (Figure 2A, 2B, 2C). The application of fresh polylysine to the culture surface provides adequate surface chemistry for the primary neuron growth both on PDL-coated glass and PDL-coated Permanox chamber slides (Figure 2E, 2F, 2G), comparable to that of the PDL-coated polystyrene culture dish (Figure 2H). On the other hand, even without the PDL coating, CC² chemically modified glass provides a superior surface for primary neuron adhesion and differentiation (Figure 2D).

Primary amines on the surface of coated and modified glass facilitate neuron attachment and survival. Previous studies have shown that neurons grown on surfaces modified with diamines and triamines resulted in good neuronal cell morphology (Corey et al., 1991, Schaffner et al., 1995, Kleinfeld et al., 1988, Spargo et al., 1994, Matsuzaqa et al., 1993, Lom et al., 1993, Healy et al., 1996). Similar observations were made with polylysine coated glass (glass slide, coverglass) and plastics (Permonox). Interestingly, by enriching the primary amines in the surface treatment, chemically modified CC² glass also supported neuronal cell growth, outperforming non-modified glass (Figure 2D) and the DAPS glass for primary neuron culture (data not shown).

Quantification of the primary amines for the biologically coated surface as well as 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	Polylysine Equivalent (g/slide)
Polylysine-coated glass	78
CC² glass	30
DAPS glass	5
Non-modified glass	<1

Conclusions

While many cells prefer culture surfaces with high surface energies (i.e. hydrophilic surfaces), neuronal cells require the additional presence of primary anime groups on the growth surface. Chemically modified CC² chamber slide surface mimics the function of polylysine coating. It provides sufficient surface chemistry (anime groups) to facilitate primary neuron adhesion and differentiation. Unlike the polylysine coating, the chemical modification of CC² is stable for more than 2 years at room temperature and requires no further coating before use. As an added benefit, the individual-seal design of the chamber slide packaging prevents contamination and preserves the integrity of the unused slides.

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