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Cell Adhesion and Growth on Coated or Modified Glass or Plastic Surfaces

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Growth substrates affect the adhesion, growth, morphology and differentiation of various cell types. However, the response to a given surface is cell type specific.

The growth of various cell types on plastic, soda lime glass and borosilicate coverglass was examined. The surfaces were either unmodified, coated with polylysine, or stably surface modified with non-biological reagents or electrical discharge. Glass surfaces were chemically modified in two ways: using a proprietary procedure and reagents or as described by Kleinfeld, et al. (1988). All substrates were assembled into Thermo Scientific Nunc Lab-Tek and Lab-Tek II Chamber Slide products.

Cells of the fibroblast-like cell line BHK-21 (baby hamster kidney), which are very adherent, did not distinguish between these growth surfaces. The less adherent fibroblast-like cell line L929 (mouse lung) and two epithelial-like cell lines, HEp-2 (human epidermal) and WISH (human amnion), grew to slightly higher densities and produced more uniform monolayers when grown on electrically modified plastic compared to unmodified plastic or glass. These cell types also grew very well on both types of chemically modified glass surfaces.

Primary brain neurons did not adhere to unmodified plastic or glass surfaces. However, neurons adhered and differentiated on polylysine coated glass or plastic surfaces and, albeit differently, on both of the chemically modified glass surfaces. Electrical modification of the plastic or glass, which significantly increased the surface energy or wettability, did not produce a surface suitable for these neurons.

Adhesion, growth and differentiation of cells on a surface is cell type specific and involves more than one mechanism. Many cell lines prefer surfaces with a high surface energy such as produced by

Lab-Tek Chamber Slide Products

	Growth surface	Well size (cm²)	Visible area (cm²)	Working volume (mL)
1 chamber	Glass/Permanox	9.4	9.4	2.5-4.5
2 chamber	Glass/Permanox	4.2	4.2	1.2-2.0
4 chamber	Glass/Permanox	1.8	1.8	0.5-0.9
8 chamber	Glass/Permanox	0.8	0.8	0.2-0.4
16 chamber	Glass	0.4	0.4	0.1-0.2
Flaskette	Glass	10.0	10.0	2.5-5.0

Lab-Tek II Chamber Slide Products

	Growth surface	Well size (cm²)	Visible area (cm²)	Working volume (mL)
1 chamber	Glass, CC2	10.0	8.6	4.0-5.0
2 chamber	Glass, CC2	4.9	4.0	2.0-2.5
4 chamber	Glass, CC2	2.4	1.7	1.0-1.3
8 chamber	Glass, CC2	1.2	0.7	0.5-0.6

Lab-Tek Chambered Coverglass

American Coverglass	Well size (cm²)	Working volume (mL)
1 chamber	9.4	2.5-4.5
2 chamber	4.2	1.2-2.0
4 chamber	1.8	0.5-0.9
8 chamber	0.8	0.2-0.4

Lab-Tek II Chambered Coverglass

European Coverglass	Well size (cm²)	Working volume (mL)
1 chamber	10.0	4.0-5.0
2 chamber	4.9	2.0-2.5
4 chamber	2.4	1.0-1.3
8 chamber	1.2	0.5-0.6

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electrical modification. Other cell types, such as primary neurons, require a specific interaction with functional groups provided by polylysine coating or chemical modification of the growth surface.

Introduction

Selection of a growth surface for cell culture should be based on cell culture performance.
Although a few cells grow in suspension, such as hematopoietic cell lines and transformed cells, most cells are anchorage dependent; that is, they grow as monolayers and require attachment to proliferate.

Historically, glass was used as the growth surface since it has superior optical qualities and is naturally charged. Disposable plastic, especially polystyrene is now most commonly used for cell culture growth. Plastic culture vessels are of good optical quality, and the growth surface is flat. However, since most plastics are hydrophobic and unsuitable for cell growth, they are often treated with radiation, chemicals or electric ion discharge to generate a charged, hydrophilic surface. Such treatment of plastic generates a surface preferred over glass by many cell types.

Growth substrates may affect the morphology, differentiation and behavior of various cell types.
A cell's repertoire on various surfaces is cell type specific. Epithelial and fibroblast cell lines remain proliferative on cell culture treated plastic. Biological coatings and chemically modified surfaces may reduce the proliferation rate by inducing differentiation into a more mature state. Cells in a more differentiated state usually function and express proteins characteristic of the tissue of origin.

Culturing neurons is a particular challenge since they do not continue proliferating after dissociation. Neuron survival in culture is dependent on cell adhesion and differentiation, which can be facilitated by modifying the surface with a biological coating or chemical modification.

This bulletin examines the correlation between primary neuron adhesion and differentiation, and surface properties such as surface energy and available specific substrate molecules.

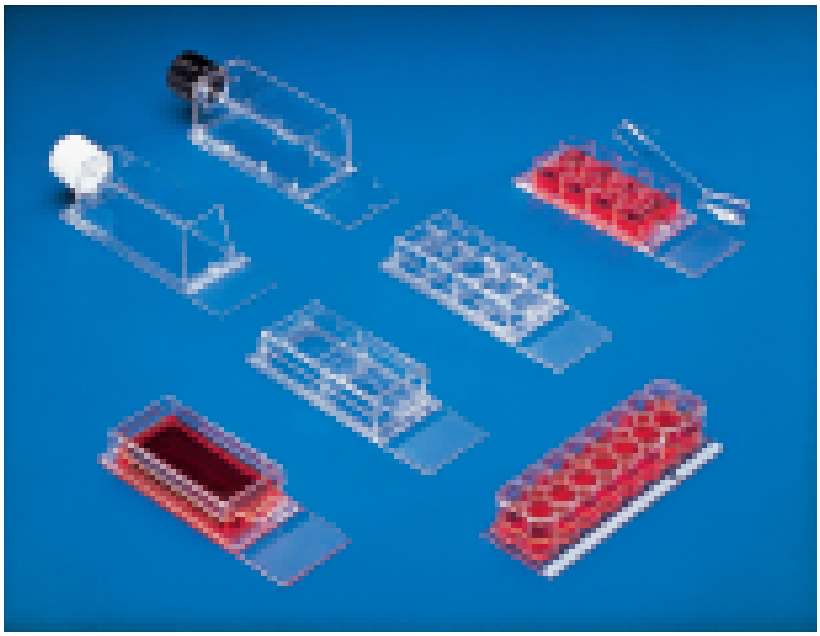
Selection of a growth surface for cell culture should also be based on application.
While glass and plastic growth surfaces are flat and optically clear they differ in many ways that may affect performance in various applications. Glass surfaces offeroptimal optical clarity with a minimal autofluorescence and are preferred for most fluorescent applications.

However, Thermo Scientific Nunc Permanox plastic slides are also acceptable for fluorescent applications. Polystyrene can be used for fluorescein if the proper blocking filters are used in the UV/blue range and perform well at longer wavelengths. Glass surfaces are more resistant than plastics to solvents, acids, bases and heat. Fixative compatibility studies have been performed on glass and plastic. Nunc™ Lab-Tek™ Chamber Slide™ products and can be found in Tech Notes No. 15 (Compatibility of Mounting Media on Thermo Scientific Nunc Permanox Slides) and No. 20 (Compatibility of Thermo Scientific Nunc Chamber Slides as Traditional Amplification Tubes). This bulletin examines cell type specific growth on each growth surface a nd some applications including fluorescence and opaque staining.

General description

Chamber Slide products are designed for the growth, fixation, staining, and microscopic examination of cultured cells on a single surface.
These products are available with a glass or plastic growth surface. Glass Chamber Slide products have a standard microscope slide manufactured from soda lime glass as the growth surface. Chamber Slide products with plastic growth surfaces are available with Permanox or polystyrene slides. These microscope slides are treated for optimal cell culture performance.

Chamber Slide products are available in two distinct designs: the original Lab-Tek design with silicone gasket and the Lab-Tek II biocompatible adhesive web design. Both have polystyrene medium



Lab-Tek Chamber Slide products

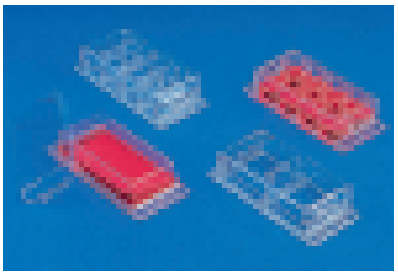
Lab-Tek II Chamber Slide products

chambers of 1, 2, 4, and 8 well formats on a slide growth surface.

The original Lab-Tek Chamber Slide design allows easy removal of the medium chamber, leaving a silicone gasket of the well format on the microscope slide, to act as a barrier between each well for differential staining. The gasket is removable allowing a cover slip to be applied.

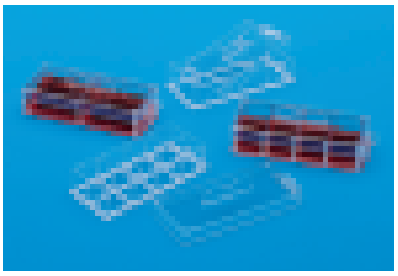
Flask style chambers offer a sealable vessel attached to a microscope slide. The Thermo Scientific Nunc Flaskette Chamber Slide product supports a flask-like medium chamber and silicone gasket on a glass growth surface. The SlideFlask medium structure is sonically welded to a polystyrene slide without a gasket or adhesive. The medium chamber easily snaps away when culturing is complete.

The Lab-Tek II Chamber Slide products have a biocompatible adhesive web design, easily distinguished by the blue mask. This design allows removal of the medium chamber and adhesive from the slide, using a component



Lab-Tek Chambered Coverglass

separation device. The blue hydrophobic border of the glass slide is raised slightly and isolates the individual wells. A cover slip can be applied.



Lab-Tek II Chambered Coverglass

Lab-Tek II Chamber Slide products are currently available with a soda lime glass growth surface. Lab-Tek II Chamber Slide products offer similar growth surface areas with larger medium volumes than the original Lab-Tek Chamber Slide products.

Lab-Tek and Lab-Tek II Chambered Coverglass products are intended for high magnification examination of living cells using an inverted microscope.
The growth surface of the Lab-Tek Chambered Coverglass products are #1 Borosilicate Coverglass of American origin. The Lab-Tek II Chambered Coverglass products are #1.5 Borosilicate Coverglass of European origin. The thin glass growth surface allows optimal optical clarity when viewing living cells with high resolution optics of an inverted microscope. The medium chambers, available in 1, 2, 4 and 8 well formats, cannot be removed from the coverglass surface.

Permanox plastic slides and all polystyrene components are made from virgin materials. All products are non-toxic and cell culture tested. Each product lot is cell performance tested to assure quality and sterility.

Methods

Cell Culture

BHK-21, a fibroblast cell line derived from baby hamster kidney (ATCC CRL 8544), was grown in BME containing L-glutamine, antibiotic/antimycotic, 10% Tryptose phosphate broth and 10% iron-supplemented bovine calf serum.

L929, a fibroblast cell line derived from adult mouse lung (ATCC CCL1), was grown in MEM containing L-glutamine, antibiotic/anti-imycotic, non-essential amino acids, and 10% iron-supplemented bovine calf serum.

WISH, an epithelial-like cell line derived from human amniocytes (ATCC CCL25), was grown in BME containing L-glutamine, antibiotic/antimycotic, and 15% iron-supplemented bovine calf serum.

HEp-2, an epithelial-like cell line derived from human epidermoid carcinoma of the larynx (ATCC CCL23), was grown in MEM

containing L-glutamine, antibiotic/antimycotic, and 10% fetal bovine serum.

PC12 cell line was derived from a rat pheochromo-cytoma (ATCC CRL 1721). The cells may be neuron-like or adrenal chromaffin-like depending on growth conditions.

PC12 cells were maintained in 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 an N2.1 defined medium (modification of Bottenstein and Sato, 1979; Bartlett and Banker, 1984) containing progesterone (20nm), putrescine (100um), selenium dioxide (30nm), 100ug/mL transferrin (bovine), 5ug/mL insulin (solubilized in 0.01N. HCl), and 0.5mg/mL ovalbumin in DMEM with 15mm HEPES (pH 7.36). Nerve growth factor (NGF; 100ng/mL) was added after four days in culture. Cells were fixed two days after the addition of NGF.

All cell lines were passaged and fed 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 10^5 cells/cm² in 10% horse serum/DMEM.

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 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 to 24 hours with filter sterilized poly-D-lysine (1 mg/mL) in borate buffer (boric acid 3.1g/L, borax 4.8g/L in water). They were washed with sterile water and dried before use.

Cell Staining
Cells were stained with crystal violet by fixing in 25% acetic acid in methanol and staining in 0.4% crystal violet.

Texas red-phalloidin stained cells were prepared by fixing in 3.7% formaldehyde in Hank's buffered saline, permeabilizing in 0.1% Triton X-100 and incubating in 1:10 dilution of Texas red-phalloidin (Molecular Probes, Inc.) in PBS.

Quantitation of Primary Amines
Primary amines were quantitated using an o-phthalaldehyde based assay (Pierce).

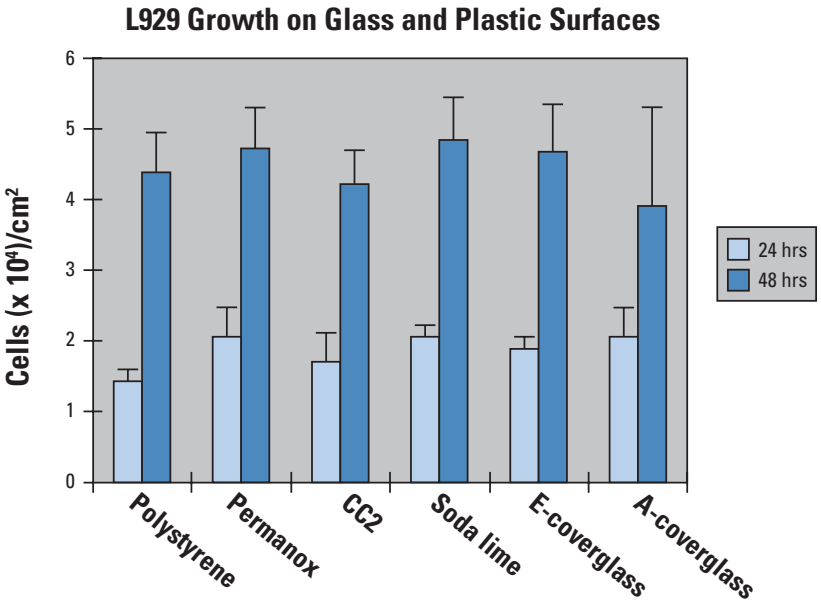
All measurements were done in soda lime glass Chamber Slide products or in Chambered Coverglass assemblies using a Bio-Tek FL500 Fluorescence Plate Reader. A fluorescence/concentration curve was generated using polylysine as the standard.

The concentration of amines on the modified surfaces are expressed in relative terms of mg polylysine/slide.

Surface energy measurement
Specific testing solutions graduated from 30-56 dynes/cm² (3DT) and distilled water with a surface energy of 72 dynes/cm² were used to determine the surface energies of the cell growth surfaces. Values were determined by brushing each solution onto the test surface and evaluating according to 3DT instructions.

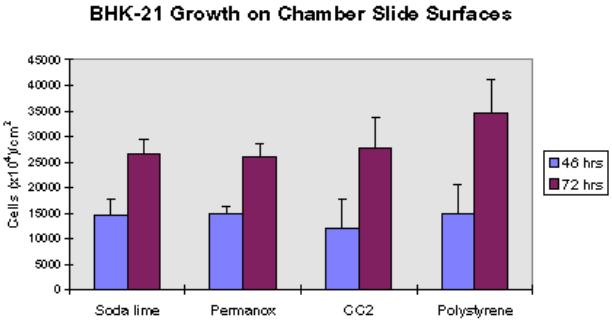
Autofluorescence measurements
Each surface was scanned with an FL500 (BioTek) using four filter pairs, i.e. excitation\emission of 360/460nm, 485/530nm, 530/590nm and 590/645nm. Since fluorescence measurements are arbitrary, sensitivity settings were adjusted so that glass readings were between 0 and 200.

Graph 1



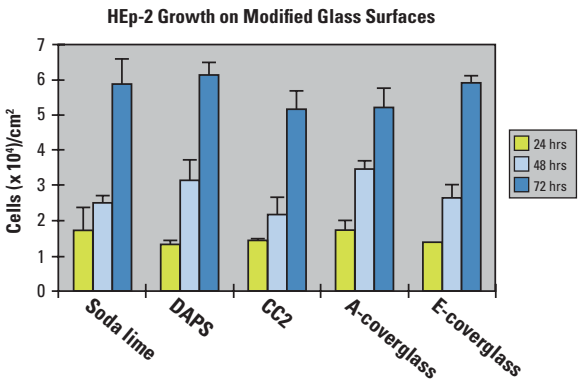
The graph shows the number of L929 cells/cm² on Chamber Slide assemblies with the indicated growth surfaces, after one and two days in culture.

Graph 2



The graph shows the number of BHK-21 cells/cm² on various growth surfaces after two and three days in culture.

Graph 3



The graph shows the number of HEp-2 cells/cm² on Chamber Slide assemblies with the indicated growth surfaces, after one, two and three days in culture.

Results and discussion

L929 cell line grew differently on plastic, glass and modified glass surfaces (Graph 1).
L929 growth curves are similar on all growth surfaces tested.

Cell morphology differs on various growth surfaces.

On both coverglass and soda lime glass, the majority of L929 cells are rounded up (Fig. 1). A few cells extend lamellapodia, becoming more spindle shaped. Cells grown on polystyrene and Permanox plastic are predominantly spindle shaped. L929 cells grown on CC2 have a more flattened, multipolar appearance than those grown on glass or plastic. This suggests that the CC2 substrate may induce changes in cell behavior, such as differentiation.

BHK-21 cell line grew differently on plastic, glass and modified glass surfaces.
BHK-21 growth on all tested growth surfaces is very similar (Graph 2).

In addition, cell morphologies of crystal violet stained BHK-21 cells look very similar when grown on glass vs. plastic (Fig. 2). However, Texas red-phalloidin staining shows very different F-actin architecture within the cells grown on glass vs. plastic. F-actin in cells grown on glass is bundled into long stress fibers whereas the F-actin in cells grown on plastic is more diffuse.

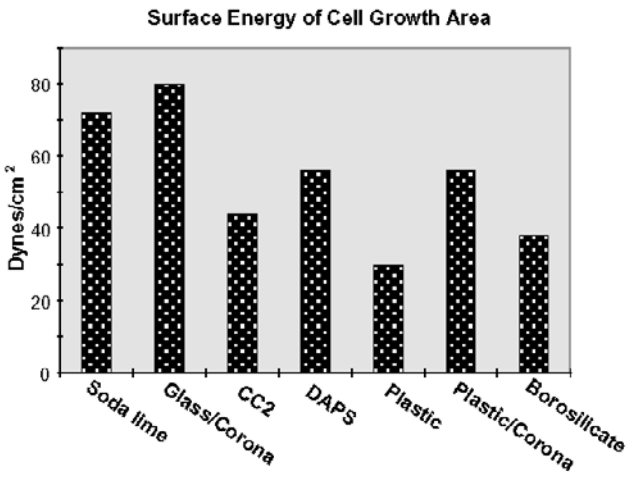
BHK-21 cells grown on CC2 modified glass display cell morphologies that are more spread and multipolar than those grown on either soda lime glass or cell culture treated plastic. This suggests that the CC2 substrate may induce changes in cell behavior.

Epithelial-like cells grew similarly on glass and plastic surfaces.
Epithelial-like cells did not show significant differences in growth or morphology on any of the surfaces tested (Graph 3 and Fig. 3).

PC12, rat pheochromocytoma cell line shows differential behavior on modified and non-modified glass.
PC12 cells appear less aggregated and more rounded when grown on CC2 modified glass (Fig. 4). Process outgrowth is most pronounced in cells grown on the European Coverglass, moderate on the CC2 and DAPS modified glass and American Coverglass, and poor on the unmodified glass. PC12 cells grown on CC2 showed fewer cell clumps and more isolated cells with round cell bodies with processes.

Glass and plastic must be adequately modified to support adhesion and differentiation of primary neurons.
Neuron adhesion and growth requires more than just the hydrophilic surface provided by glass and cell culture treated plastic (Fig. 5). Very few neurons survive on bare glass or cell culture treated Permanox. The application of fresh polylysine to the surface immediately before use, provides adequate surface chemistry for neuron growth. CC2 modified soda lime glass without polylysine

Graph 4



also provides a good surface for primary neuron adhesion and differentiation. CC2 surface is stable for more than two years at room temperature and requires no further coating before use. While polylysine-coated European Coverglass provides a good surface for neuronal adhesion and differentiation, polylysine-coated American Coverglass does not support neuronal adhesion and survival.

Surface Energies of the Modified and Unmodified surfaces.
The surface energy of soda lime glass is very close to 72 dynes/cm² (Graph 4).

Chemical modification of glass results in decreased surface energies. For example, DAPS treatment decreases the surface energy of glass from 72 to 56 dynes/cm²; the surface energy decreases further over the next two days (not shown). Plastics are generally hydrophobic in nature as shown by the low surface energy. Corona treatment increases the surface energy of plastic, thus producing a hydrophilic surface.

The surface energy of borosilicate coverglass is lower than that of soda lime glass.

Primary amines on the surface of coated and modified glass may facilitate neuron attachment and survival.
It was previously shown that neurons grown on surfaces modified with diamines and triamines formed cultures whose cell morphologies were similar to those on polylysine coated glass (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). Therefore primary amines were quantified on each of the surfaces to determine if this correlated with neuron adhesion (Table 1).

The modified surfaces express levels of primary amines very similar to that of polylysine coated surfaces, which correlates well with their ability to support neuron adhesion and differentiation.

Autofluorescence of Chamber Slide Growth Surfaces.
For fluorescent applications (Graph 5) such as Fluorescent In Situ Hybridization and Immuno-fluorescence, it is important that the growth surface is non-autofluorescent at the wavelength used for fluorochrome detection. Glass slides or coverglass are most often used for these applications. This graph shows that Permanox performs as well as glass with the most commonly used fluorochrome wavelengths. Though polystyrene performs poorly in the UV/Blue range (360/460nm) it performs as well as or better than glass at longer wavelengths.

Conclusions

The effect of growth surfaces on adhesion, growth and differentiation of cells is cell-type specific and involves more than one mechanism.

- Many cells prefer surfaces with high surface energies (i.e. hydrophilic surfaces).
- Growth surfaces such as CC2 may improve cell adhesion and induce cellular differentiation of fibroblastic-like cells.
- High surface energy is not sufficient for primary neuron growth.
- Biological coatings or chemical modifications that place amines on the growth surface facilitate primary neuron adhesion and differentiation.
- Other variables, such as those that differentiate European and American borosilicate coverglass, may influence neuron survival on glass surfaces.

Table 1

Quantification of Primary Amineson Coated and Modified Glass Surfaces

Treatment	Polylysine Equivalent
Polylysine/Soda Lime	82µg
Polylysine/E-Coverglass	74µg
Polylysine/A-Coverglass	77µg
CC2	30µg
DAPS	5µg

Graph 5

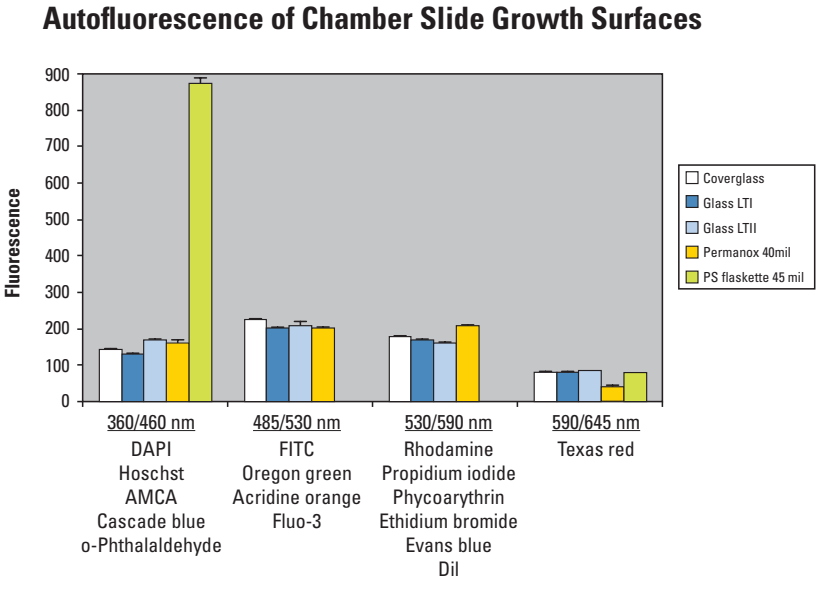
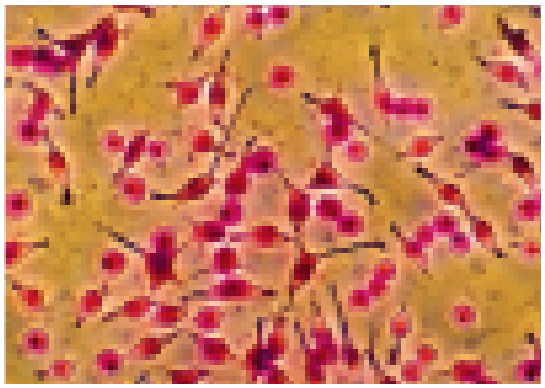
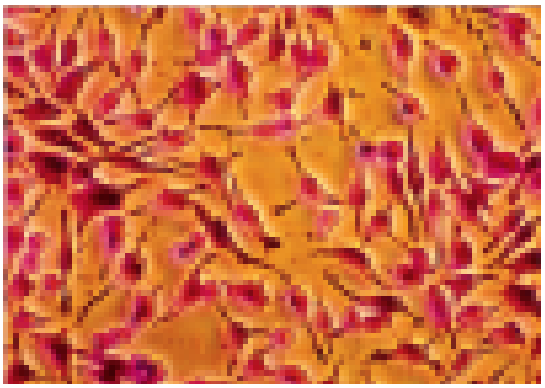


Fig. 1

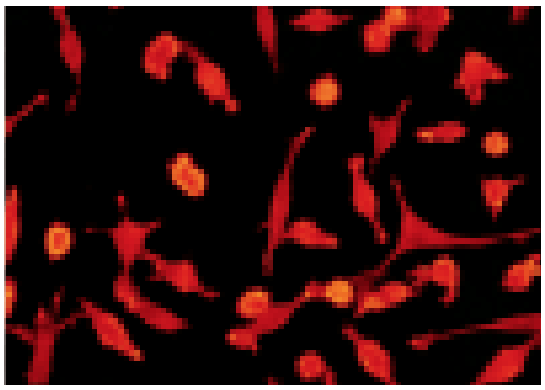
L929 Growth on Plastic and Glass Surfaces



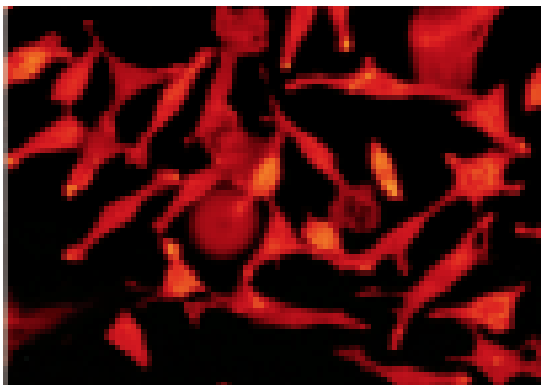
Coverglass



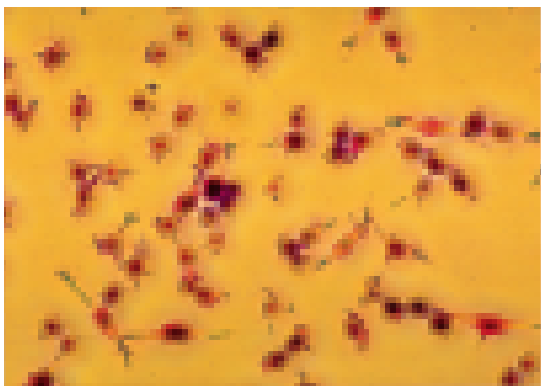
Polystyrene



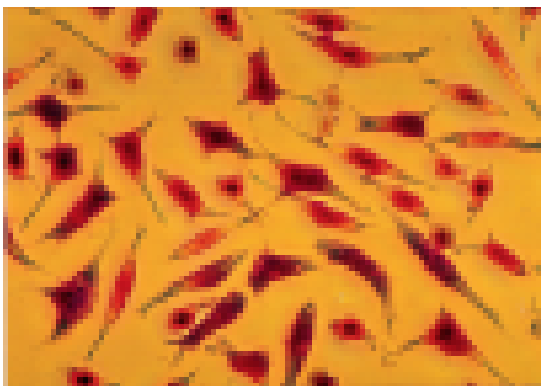
Soda Lime



Permanox



Soda Lime

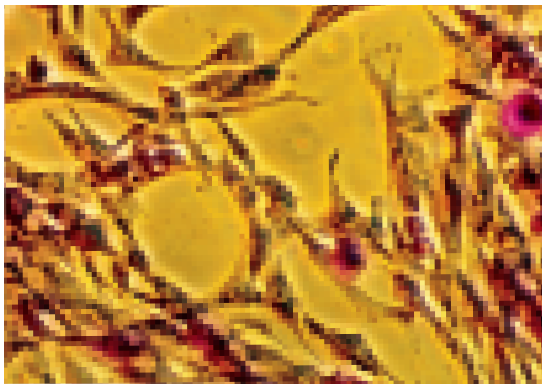


CC2

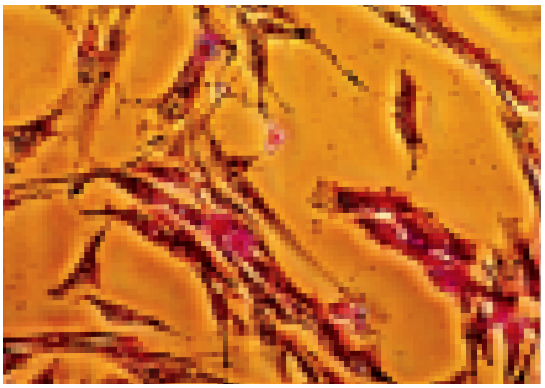
- The top two panels show crystal violet stained L929 cells grown on coverglass (left) and cell culture treated polystyrene (right).
- The middle panels show cells grown on soda lime glass (left) and Permanox plastic (right) and labeled with Texas red-phalloidin.
- The bottom two panels show crystal violet stained L929 cells grown on soda lime glass (left) and CC2 modified glass (right).

Fig. 2

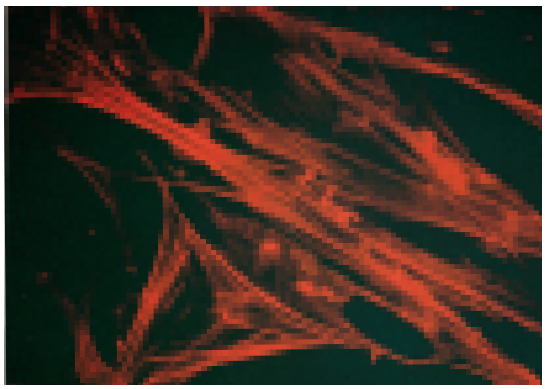
BHK-21 Growth on Plastic and Glass Surfaces



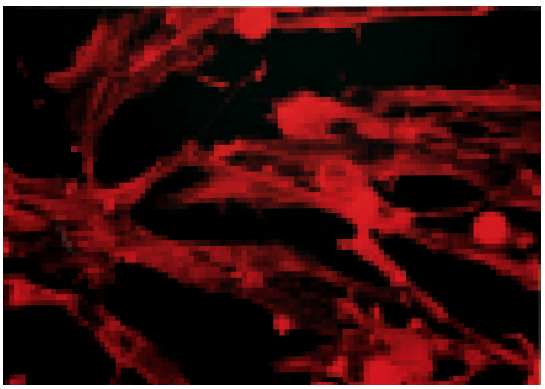
Coverglass



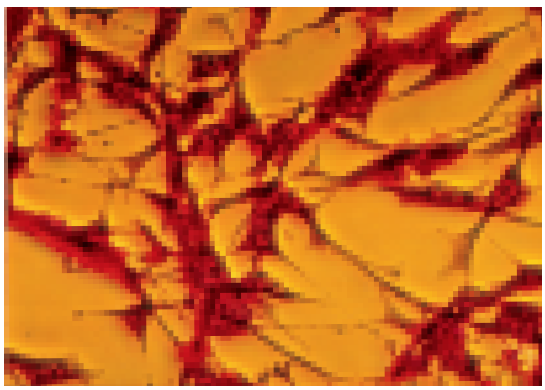
Polystyrene



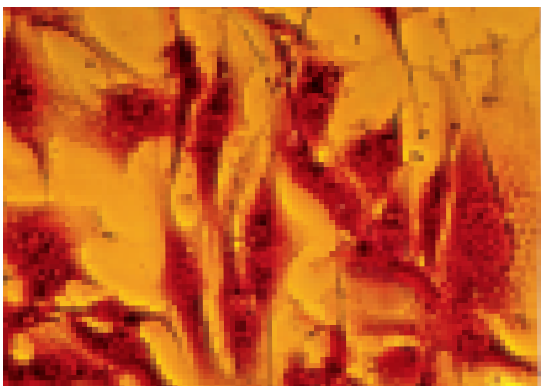
Soda Lime



Permanox



Soda Lime



CC2

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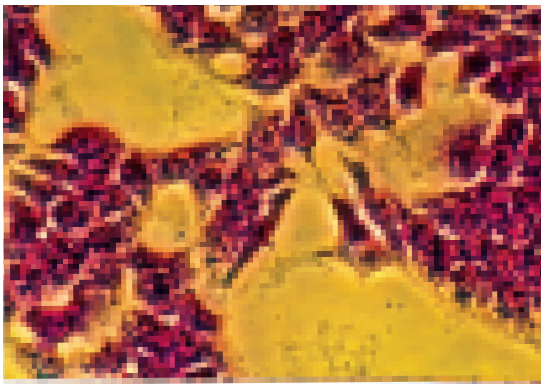
- The bottom two panels show crystal violet stained L929 cells grown on soda lime glass (left) and CC2 modified glass (right).

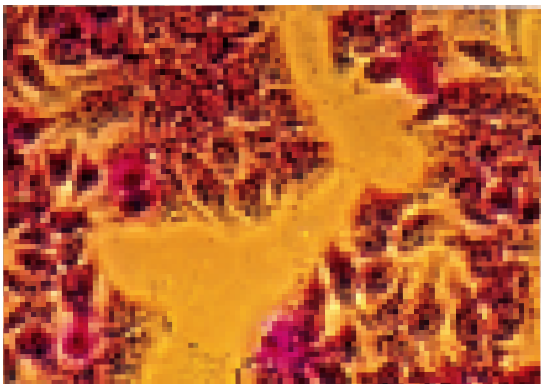
Fig. 3

Epithelial-Like Cell Growth on Plastic and Glass Surfaces

WISH

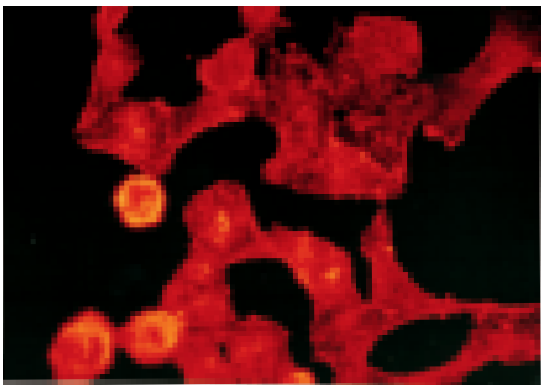


Coverglass

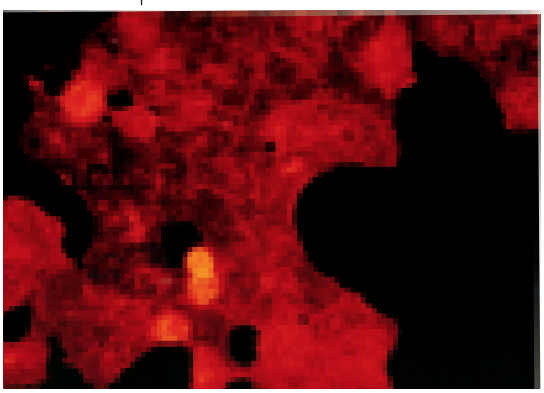


Polystyrene

WISH

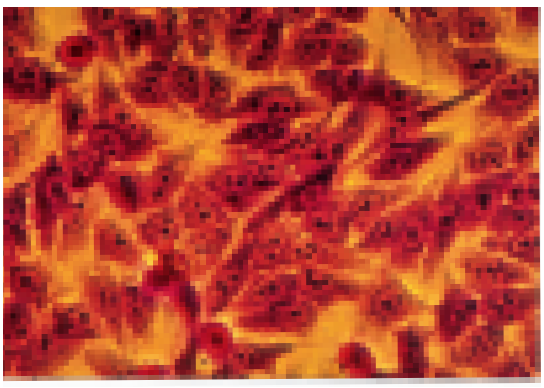


Soda Lime



Permanox

HEp-2



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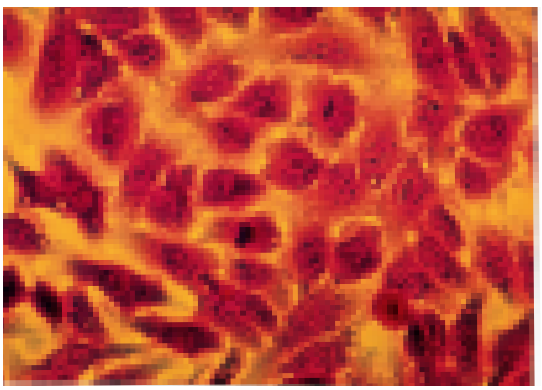
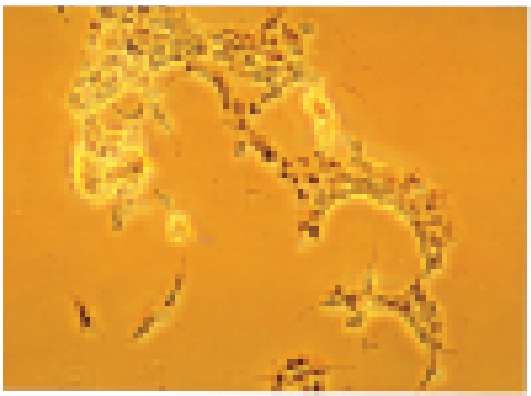


CC2

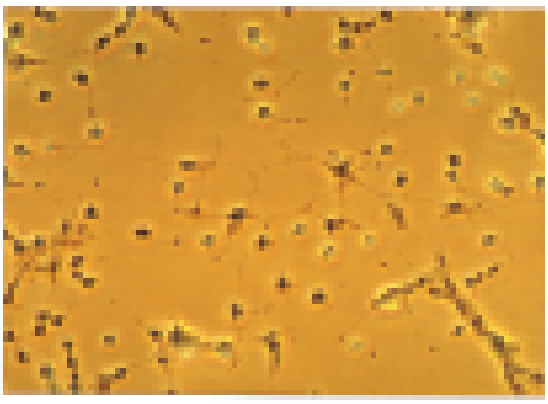
- The top two panels show crystal violet stained WISH cells grown on coverglass (left) and cell culture treated polystyrene (right).
- The middle panels show WISH cells grown on soda lime glass (left) and Permanox plastic (right) and labeled with Texas red-phalloidin.
- The bottom two panels show crystal violet stained HEp-2 cells grown on soda lime glass (left) and CC2 modified glass (right).

Fig. 4

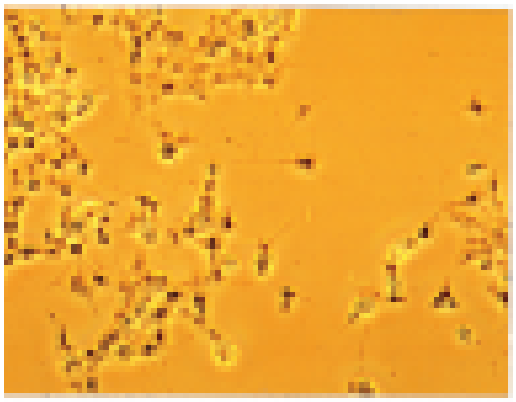
PC12 Growth On Modified Glass



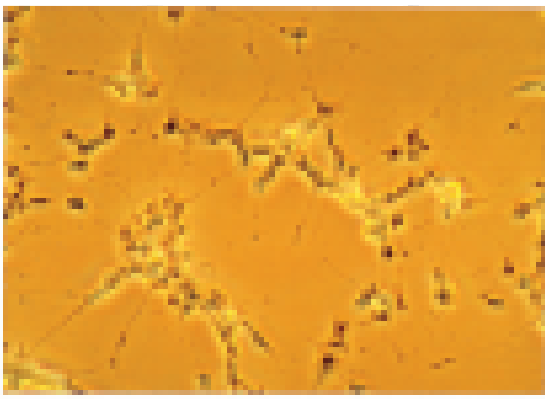
Not modified



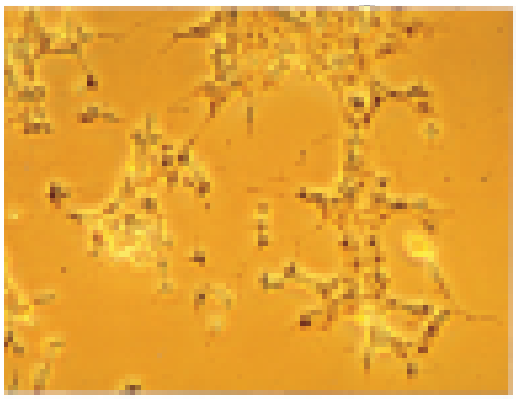
CC2



DAPS



European Coverglass

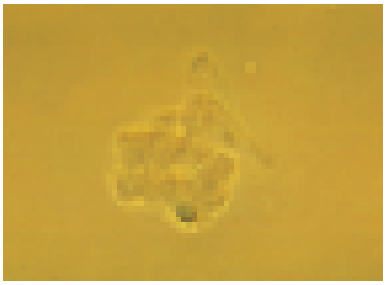


American Coverglass

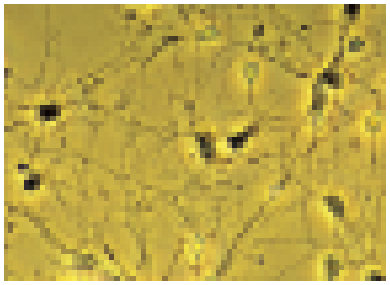
- PC12 cell line, grown in serum free defined medium with added NGF, is shown plated on unmodified soda lime glass, CC2 modified soda lime glass, DAPS modified soda lime glass and European and American type borosilicate coverglass.
- No polylysine was used.

Fig. 5

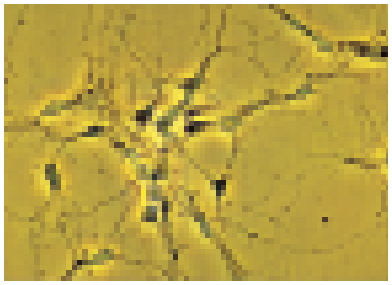
Primary Chick Neuron Growth on CC2 and Coated Coverglass and Plastic



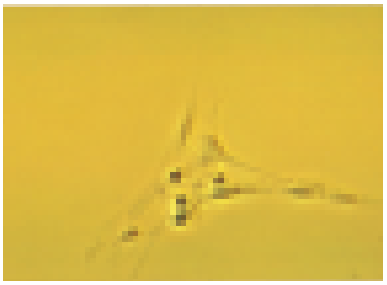
Permanox



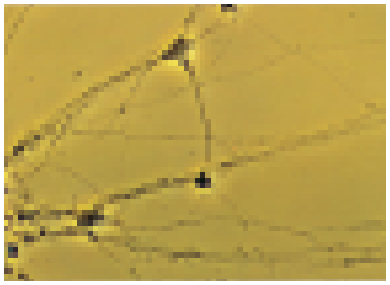
Permanox/Polylysine



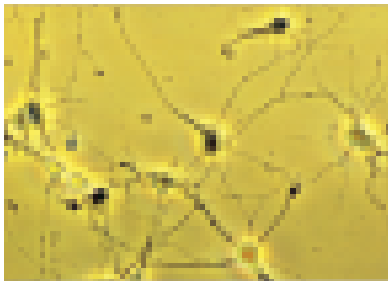
Polystyrene/Polylysine



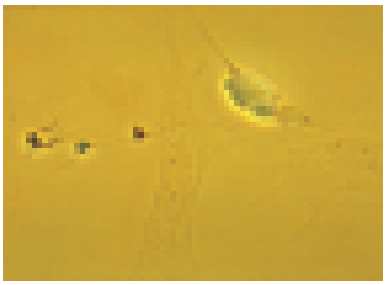
Soda Lime



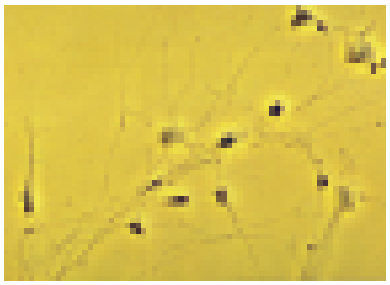
Soda Lime/Polylysine



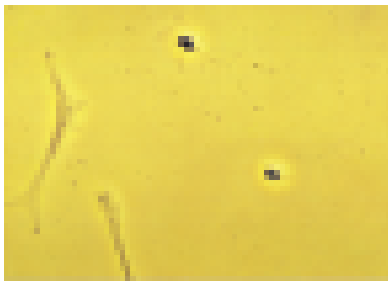
CC2



E-Coverglass



E-Coverglass/Polylysine



A-Coverglass/Polylysine

- The left column shows primary neuron growth on cell culture treated Permanox plastic (top), soda lime glass (middle) and European borosilicate coverglass (bottom).
- The middle column shows neuron growth on Permanox plastic, soda lime glass and European borosilicate coverglass, each with a pre-coating of polylysine.
- The right column, first row shows neurons grown on polylysine coated polystyrene.
- The right column, middle row shows neuron growth on a CC2 modified surface, without polylysine.
- The right column, bottom row shows neurons grown on polylysine coated American coverglass.

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