forum
No. 12, 2011
Technical Notes and Applications for Laboratory Work

Content

Advanced TC™: An innovative surface improving cellular assays

1. Introduction

Cellular assays and screening approaches using immortalised cell lines, primary cells or co-culture models are nowadays one of the most important biotechnological tools used in basic research. Scientists in both the academic and industrial arenas are increasingly adopting a cell-based approach, shifting their focus away from biochemical analysis of discrete cellular components to the complex analysis of an *in vivo*-like system[1]. The utilisation of living cells leads to more comprehensive data, which complements and enhances results gained from biochemical assays.

1.1 Obstacles of in vitro cell culture

Cell cultivation and propagation *in vitro* can be challenging. *In vivo* cells of a multi-cellular organism are embedded in the three-dimensional structure of the extracellular matrix (ECM) of adjacent cells. In addition to providing structural support, the ECM also comprises a wide range of cellular growth factors and mediates biochemical signals which essentially influence cellular proliferation and survival[2,3].

greiner bio-one

Cultivation of cells *in vitro* usuallly refers to a two-dimensional culture on plastic surfaces which lack the vital signals provided by the connective tissue. A possible solution to counterbalance this deficit is the addition of fetal calf serum (FCS) to the respective cultivation media. FCS contains various growth factors supporting cellular proliferation and serum proteins which can bind to the plastic surface and create an ECM-like surface promoting cellular adhesion. Due to possible variations of serum composition and the potential contamination risk based on the biological origin of the FCS, pharmaceutical and biotechnological companies nowadays favour a serum-reduced or serum-free cultivation medium. These restricted growth conditions however minimise protein binding to the cultivation surface and cellular adhesion respectively. The absence of growth factors also results in decelerated proliferation rates. Therefore the required usage of chemically defined and FCS-free media can lead to variable cell loss, experimental inconsistencies and delayed test results due to extended cultivation periods and assay repeats.

Beside immortalised cell lines, researchers can also face problems with primary cells, which have gained importance for biotechnological research in the last couple of years. Primary cells are cells explanted from original tissue of the host organism and react in a more native way when compared to immortalised cell lines. Mimicking the organ from which the cells were originally derived these systems are prized by researchers but conversely are difficult to culture and maintain. The need of an *in vivo*-like environment and a limited life span requires the adaption of the media composition or the respective cultivation surface.

Comparable to primary cells, co-culture models facilitate insight into complex interactions in a tissue-like assembly. Their cultivation can be extremely complicated as not only the requirements of a single cell type but of a combination of cells has to be accommodated. Preserving *in vitro* the cellular interconnection and function during this type of culture procedure is extremely demanding. Researchers are not only confrontated with drawbacks due to characteristics of the respective cell type, they also have to keep pace with upcoming future technologies. During the last decade biotechnology has become a fully integrated industrial process leading to an increasing need for automated solutions.

These types of assays, categorised as high-throughput screening (HTS) or high-content analysis (HCA) facilitate comprehensive cellular examination. The use of robotics in these fields often results in increased cell stress or cell loss during automated washing steps and media changes. Irregular cellular attachment caused by common variables in cell culture can lead to assay inconsistencies and higher variances between individual assays. This can minimise the significance or informative value of the performed experiment. In summary, the developments in biotechnology and the obstacles involved with *in vitro* cell culture described above lead to the conclusion that there is a need for the optimisation of current cultivation platforms in order to address these issues.

1.2 Tissue culture surfaces

1.2.1 Physical surface treatment

In general, cell culture disposables made from plastic, like polystyrene, need to be surface treated in order to create a hydrophilic surface and to facilitate effective cell attachment. Physical surface treatments typically involves the use of directed energy at the substrate surface with the intention of generating chemical groups on the polymer surface. Such chemical groups include amines, amides, carbonyls, caboxylates, esters, hydroxyls and the like. Atmospheric corona and vacuum plasma treatments are commonly used for polymeric surface activation for cell growth substrates as well as medical implants. The increased hydrophilicity and wettability results in improved cell spreading and attachment even of cells that may have adhered poorly due to their cell phenotype or stressful culture conditions.

Besides this effect, atomic force microscopy reveals that in contrast to a smooth native polystyrene surface, tissue culture surface treatment leads to the formation of surface spikes and an overall roughness which in addition to the creation of a hydrophilic surface leads to enhanced cell adhesion[4, 5].

1.2.2 Biological coated surfaces

Inside a tissue, cells are in constant contact with neighbouring cells and the extracellular matrix (ECM). The ECM is a complex aggregation of molecules consisting of proteoglycans and insoluble fibrous proteins. Interactions between cells and the ECM components are important in many biological processes such as cell growth and differentiation[6]. In general cells utilise transmembrane glycoproteins to attach to specific cell binding sites that are displayed by ECM proteins such as collagen, fibronectin and laminin. Adsorption or immobilisation of such ECM proteins can therefore be used to enhance cell attachment to cell culture plastic disposables.
However this approach has some limitations. The surface coating process is difficult to control and adsorption might lead to denaturation of the protein resulting in reduced functionality. Orientation and density of the protein can influence effectiveness of cellular attachment implying batch inconsistency and reduced reproducibility.
To overcome these obstacles Greiner Bio-One offers the protein coated CELLCOAT® products manufactured with minimal batch-to-batch variability and known composition and protein activity.

1.2.3 Advanced TC™

Another approach is the imitation of the cellular surrounding based on a physical-chemical process excluding the utilisation of biological proteins. Based on this idea Greiner Bio-One has developed the novel Advanced TC™ cell culture products.
The production process assures constant and reproducible product quality and facilitates sterilisation of the final end product as well as transport and storage at room temperature. Due to the non-biological origin the surface is not susceptible to degradation and is stable for several years.
In addition, the possibility of cross reaction or contamination based on a biological protein coating can be circumvented.

Beside these concomitant product features the Advanced TC™ technology has a preeminent effect on cell cultivation processes:
Advanced TC™ facilitates consistent and homogenous cell attachment, increasing the overall cell yield and reducing cell loss for example during automated washing steps of high-throughput applications.
Enhanced cell attachment (see chapter 2.2 / 2.3) and higher proliferation rates (see chapter 2.1) improve and accelerate cell expansion, facilitating in particular cultivation of fastidious cells as well as cells cultivated under restricted growth conditions. Compared to classical tissue culture surfaces Advanced TC™ optimises primary and long-term adhesion leading to a native, *in vivo*-like morphology (see chapter 2.2 / 2.3) and better cell differentiation (see chapter 2.5).
This positive morphological effect is particularly apparent during cultivation of primary and sensitive cells, serum deprivation or after cellular stress induced by transfection or transduction processes. Positively influencing cellular features and functions, Advanced TC™ also leads to higher transgene activity after the described gene transfer (see chapter 2.4).

Figure 1: Advanced TC™ product familiy

2. Optimising cellular assays with the innovative Advanced TC™ surface

2.1 Cell proliferation

Cell-based assays nowadays play an important role in life science and drug discovery, especially in secondary screening studies of promising drug compounds. Advances in a number of technologies have made the utilisation of live cells in high-throughput (HTS) and high-content (HCS) screening an attractive adjunct to biochemical and other assays used in the drug discovery process. Automation of these assays requires a steady stream of cultured cells. Therefore cultivation of cells on the improved Advanced TC™ surface leading to higher proliferation rates and the rapid attainment of maximum cell numbers can guaranty constant availability of cells with consistent quality. Besides high-throughput and high-content assays, optimised and accelerated cell expansion can also positively influence biopharmaceutical processes like vaccine or antibody production.

To analyse cellular proliferation four different cell lines have been cultivated on the CELLSTAR® TC, the novel Advanced TC™ and two improved competitive surfaces either in complete growth medium (CHO, HepG2, SK-N-MC) or under serum-free conditions (HEK 293).
Cells were pre-cultivated in tissue culture flasks, harvested by trypsin/EDTA digestion and thereafter seeded in identical cell concentrations on 96 well plates from the respective manufacturers. Cells were allowed to grow for 72 hours and the number of viable cells was determined each day by MTS assay: The reagent 3-(4,5-dimethylthiazol-2-yl)-(3-carboxy-methoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) is reduced by the cellular mitochondrial dehydrogenase of metabolically active cells into a formazan product that is soluble in tissue culture medium.
Therefore the absorbance of the formazan product can be measured and quantified directly at 490nm from 96 well plates without additional processing. The quantity of the formazan product is directly proportional to the number of living cells in culture.

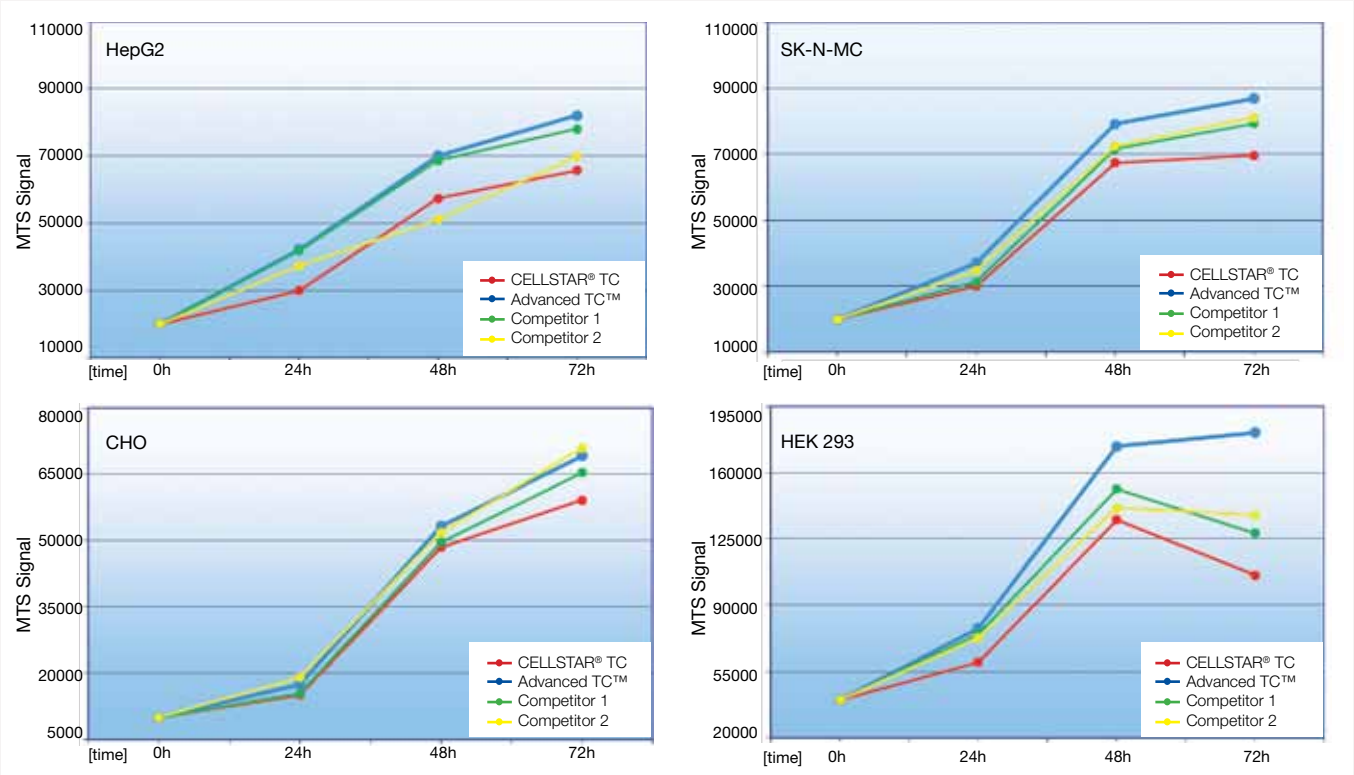


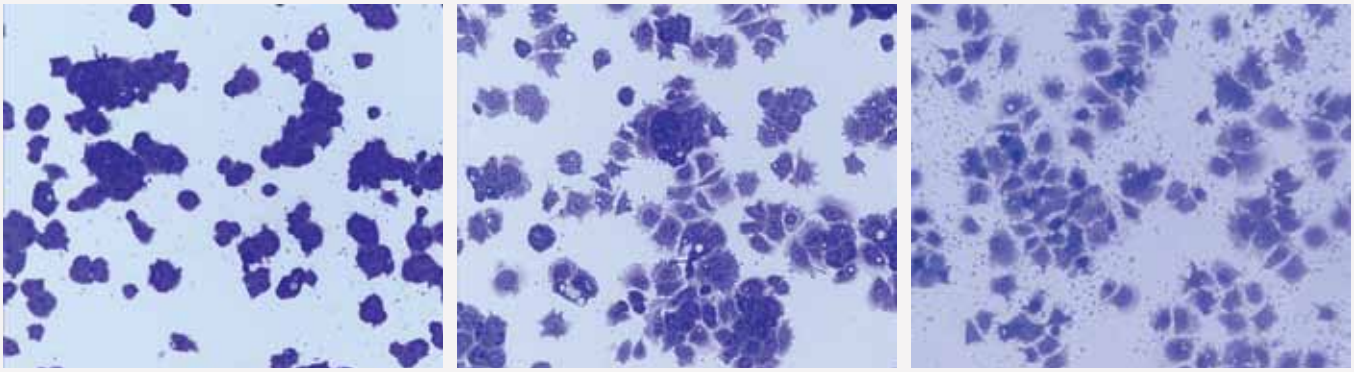
Figure 2: Cell proliferation of HepG2, SK-N-MC, CHO and serum-free cultivated HEK 293 cells

As shown in figure 2 Advanced TC™ induced a distinct increase in cell growth and proliferation for all tested cell lines. Compared to the standard tissue culture surface cultivation on the improved surface lead to 24,8% more cells after 72 hours for HepG2 and SK-N-MC, 16,8% for CHO and 71,1% for HEK 293. The massive increase for the latter cell line cultivated under serum free conditions is due to a vast cells loss on the TC-treated surface during the media change after 48 hours indicated by a steep decline of the red curve. In the absence of serum, growth factors supporting cellular expansion as well as serum proteins linked to the plastic surface promoting cellular adhesion are missing leading to loosely attached cells. Under these conditions shear forces applied by pipetting of fluids can cause cellular detachment and irrevocable cell loss.
Improved cell attachement on the physically-chemically-modified Advanced TC™ surface (see also chapter 2.2/ 2.3) maintains a cohesive cell layer and minimises cell detachment during media changes and washing steps.

2.2 Primary and long term adhesion

Cellular attachment kinetics follows a sigmoidal curve with a lag phase prior to cell attachment due to the time taken for cells to sediment to the bottom of the cell culture vessel. Teare et al.[2] demonstrated a direct correlation between the hydrophilicity of the surface and the rate of cell attachment, where cellular adhesion is more rapid as the hydrophillicity of the surface increases.

Depending on the type of media used, this process is either reliant on direct interaction of the cell with the respective surface (serum-free media) or the adsorbed protein pattern. This will ultimately affect the number of cells attached and the strength of adhesion[7]. Methods for quantifying the number of attached cells include direct microscopic visualisation or colorimetric assays such as crystal violet staining.
Crystal violet is a protein dye which stains cells with a deep purple colour. Attached cells are fixed with methanol contained in the staining solution and thereafter excessive dye is removed by several washes with distilled water. Due to these rigorous washing steps the crystal violet staining can also be used to determine the strength of cellular adhesion.
For primary adhesion analysis, cells were seeded in their respective growth media and stained with crystal violet after two, four, six and eight hours.
After two hours HepG2 cells already exhibit their typical *in vivo* morphology and a high degree of differentiation on the Advanced TC™ surface whereas cells on the control plate reach this status at around eight hours. Therefore the Advanced TC™ surface leads to a much more rapid cellular attachment as well as differentiation of these liver cells similar to cells cultivated on CELLCOAT® PDL (Fig. 3). Hence for HepG2 cells the Advanced TC™ polymer modification is comparable to a protein-coated surface imitating the native, ECM like surrounding of the cells.



CELLSTAR® TC | Advanced TC™ | CELLCOAT® PDL

Figure 3: Primary Adhesion of HepG2 cells after 2 hours

An equivalent effect is visible for the cell line SK-N-MC and serum-free cultivated HEK293 cells: First indications of a neuronal phenotype can be detected for SK-N-MC cells after four hours when cultivated on Advanced TC™ whereas HEK 293 cells display a higher proportion of attached and flattened cells at this time point (Fig. 4).

This indicates that similar to HepG2 a non-biological surface like Advanced TC™ can positively influence processes which are essential for cellular survival. Based on the faster and stronger attachment, cells can initiate earlier cell proliferation and differentiation.

The highly proliferative cell line CHO, having only minor demands on their required growth substrate, displays no visual effect after four hours in culture (Fig. 4). The number of cells remaining on the surface after the crystal violet staining as well as their respective morphology is identical on all tested surfaces. But differences could be detected after longer cultivation periods.

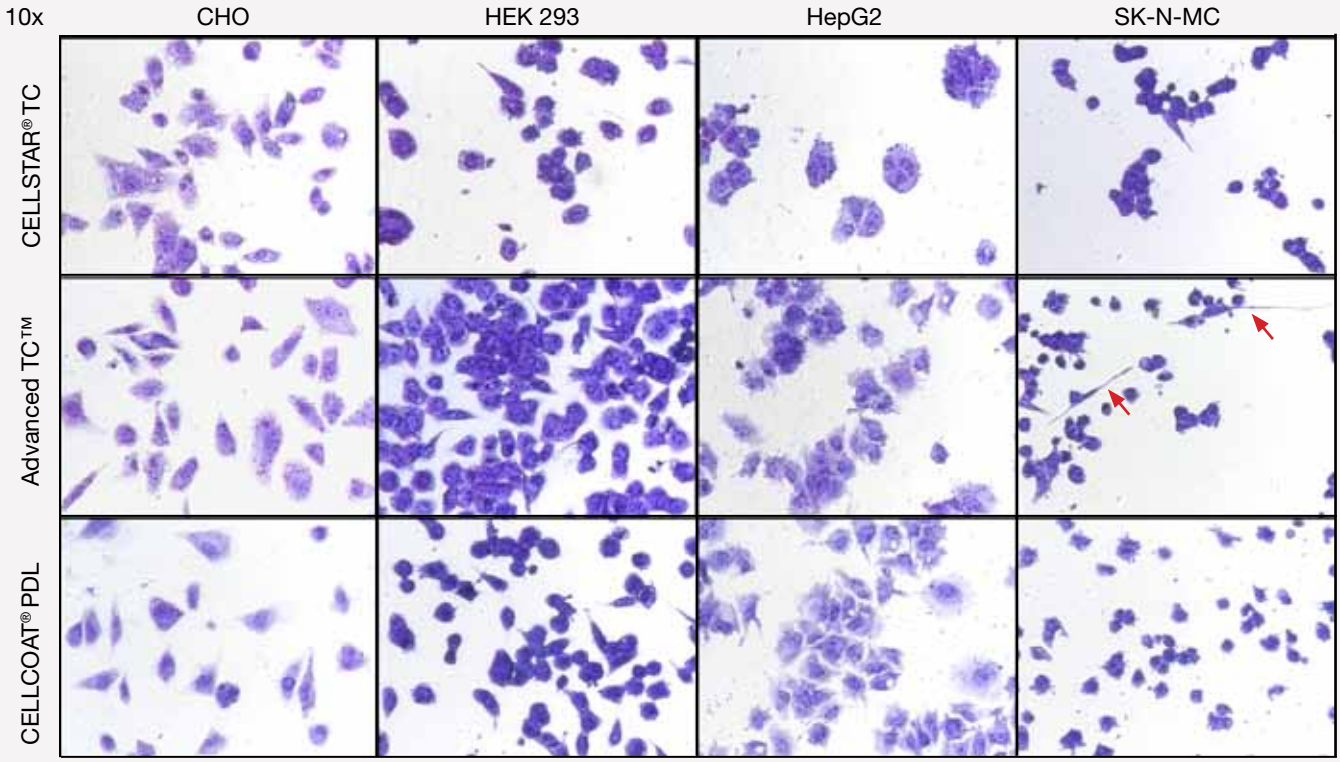


Figure 4: Primary Adhesion after 4 hours of CHO, HEK 293 HepG2 and SK-N-MC cells (40x magnification). Arrows indicate neuronal phenotype of SK-N-MC cells.

Long term adhesion was compared after 48 hours of cultivation by crystal violet staining. Comparing the intensity of the staining on the three tested surfaces reveals slightly higher cell numbers for CHO cells on the novel Advanced TC™ surface. Likewise the protein coated surface leads to stronger cell attachement and higher profliferation rates when compared to CELLSTAR® TC. The analysis of the sensitive cell lines (HepG2, SK-N-MC and serum-free cultivated HEK 293 cells) revealed not only differences in cellular adhesion strength but also morphological changes. After 48 hours all three cell lines feature an evenly distributed cell layer with a high number of cellular interconnection on the Advanced TC™ surface.

In parallel, cells cultivated on standard tissue culture surfaces display less intense cell attachment, cell clumping or cell loss after the required washing steps. A major difference was visible for SK-N-MC characterised by a high number of differentiated neuronal cells when cultivated on the Advanced TC™ polymer modification. Similarly HepG2 cells maintained their high degree of differentiation on the non-biological surface while the standard tissue culture surface leads to cell detachment and clumping (Fig. 5).

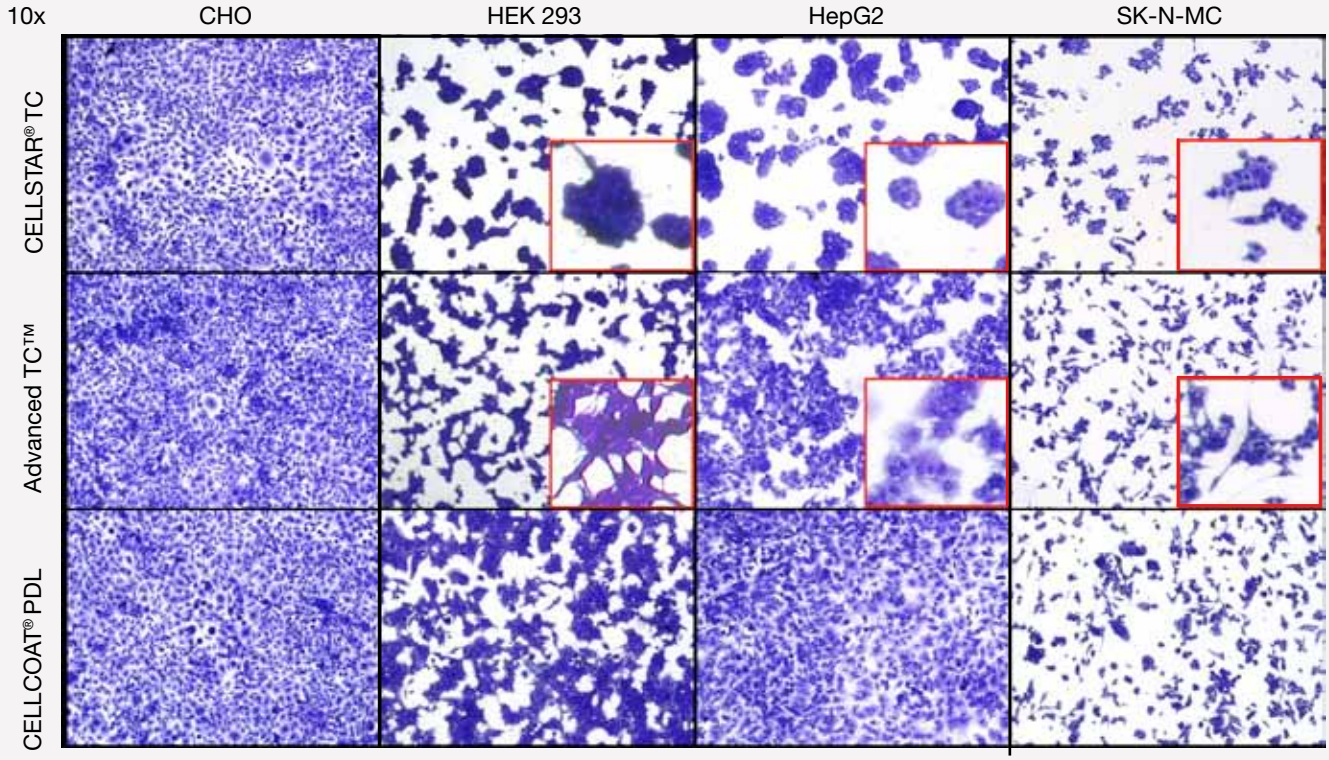


Figure 5: Long-term adhesion after 48 hours of CHO, HEK 293, HepG2 and SK-N-MC cells (40 x magnification). Red framed magnifications display cell specific details of CELLSTAR® TC and Advanced TC™.

2.3 Expression of adhesion proteins

In vitro and *in vivo* cell adhesion is essential for the assembly of individual cells into a tissue like structure. Cell adhesion molecules and adhesion receptors usually consist of transmembrane glycoproteins, such as cadherin at cell-cell contact and integrin at cell-matrix contacts. The extracellular matrix proteins are large peripheral membrane proteins that are associated to adhesion receptors at the intracellular surface of the plasma membrane and form a linkage to the actin cytoskeleton[9].

Most cultured and stationary cells adhere tightly to the underlying growth substrate through distinct regions of their plasma membrane known as focal adhesion plaques, focal contacts or focal adhesions (Fig. 6). At these sites, transmembrane receptors interact with extracellular matrix proteins e.g. fibronectin, collagen, laminin or vitronectin. On the cytoplasmic side of focal adhesion plaques, cytoskeletal proteins link large bundles of microfilaments to these structures. Thus the focal adhesions are structural connections between the ECM and the cellular actin cytoskeleton.

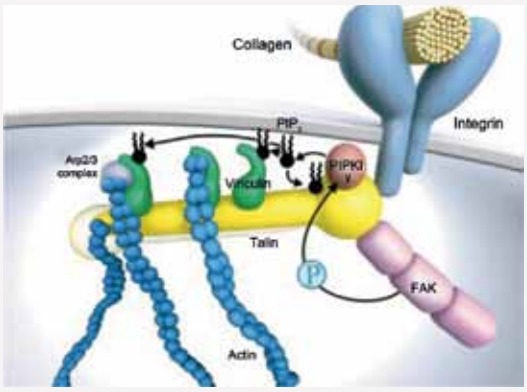


Figure 6: The focal adhesion plaque as a complex of intergrin, adhesion proteins and actin filaments[9].

In vivo cell adhesion occurs in three phases: attachment, spreading and formation of the described focal adhesion plaques (Fig. 7). The first and reversible step of weak cell attachment involves the interaction of integrins and the growth substrate.

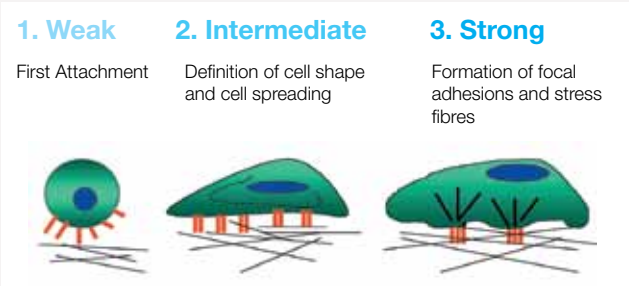


Figure 7: Three phases of cell attachement[10].

Binding of ECM components to the integrin molecule induces integrin clustering and increases integrin affinity for the ligand. A process known as integrin activation. Following these initial cell-receptor-ECM ligand interactions, the cell increases its surface contact area with the ECM substrate through formation of actin microfilaments and cell spreading. This stage of attachment is considered an intermediate state between that of weak contact and strong adhesion and is essential for cell survival and differential gene expression.

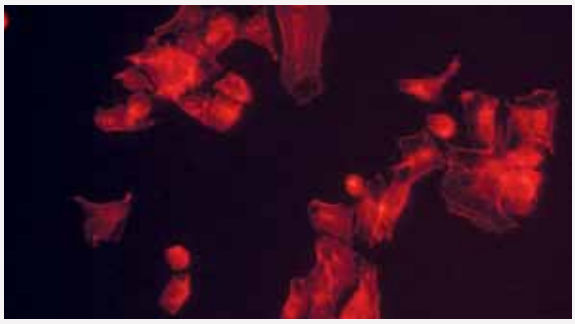
If the appropriate signals are provided by the matrix, cells proceed to organise their cytoskeleton by the formation of focal adhesion plaques and actin containing stress fibres[10] inducing cell differentiation and leading to stationary cells and a tissue like assembly.

In contrast to the *in vivo* situation, cells cultivated on polymer surfaces can not build on adhesive signals provided by the surrounding matrix.

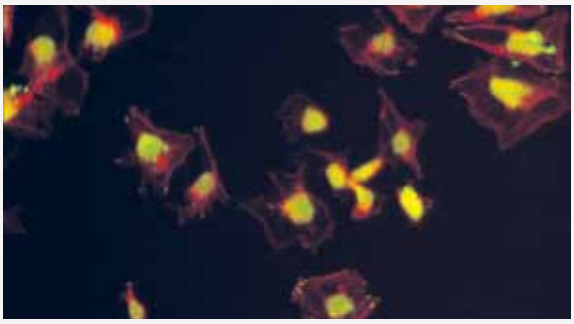
Nevertheless studies of Keselowsky et al.[8] revealed that chemical surface modifications can modulate focal adhesion composition and influence cell attachment through changes in integrin binding.

Neutral hydrophilic -OH functional groups supported the highest level of recruitment of adhesion proteins such as talin, actinin and paxillin to adhesive structures. Positively charged $-NH_2$ and negatively charged -COOH surfaces exhibited intermediate levels of recruitment of focal adhesion components, while a hydrophobic $-CH_3$ substrate displayed the lowest level.
The pattern in focal adhesion assembly correlated with integrin binding and cellular attachment. Therefore there is major evidence that a chemical micropatterning can lead to improved cell adhesion as can be demonstrated by the novel Advanced TC™ surface:

To analyse cellular adhesion and expression of relevant adhesion proteins, cells were cultivated on various growth substrates, fixed at specific time points and stained for vinculin, a member of the focal adhesion plaque complex and actin containing stress fibres. Even though CHO cells displayed less prominent effects for cell proliferation and general cell adhesion, formation of focal adhesion plaques differed significantly on the Advanced TC™ and standard tissue culture surface. After two hours, expression of vinculin (Fig. 8) and the specific accumulation at the cell-surface interaction site (Fig. 9) could already be detected whereas cells on the CELLSTAR® TC surface displayed no vinculin staining at these timepoints.

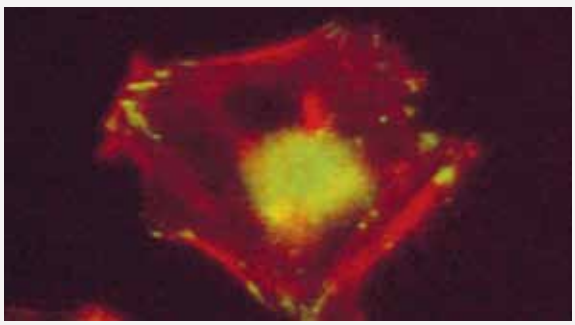


CELLSTAR® TC

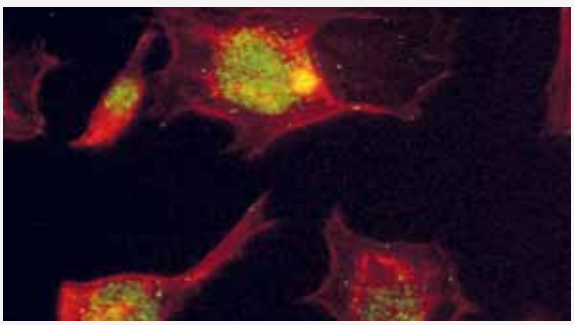


Advanced TC™

Figure 8: Staining for vinculin (green) and actin containing stress fibres (red) in CHO cells after 2 hours of cell attachment.



2 hours



4 hours

Figure 9: Detailed view of CHO cells stained for vinculin (green) and actin containing stress fibres (red) after 2 and 4 hour cultivation on the Advanced TC™ surface.

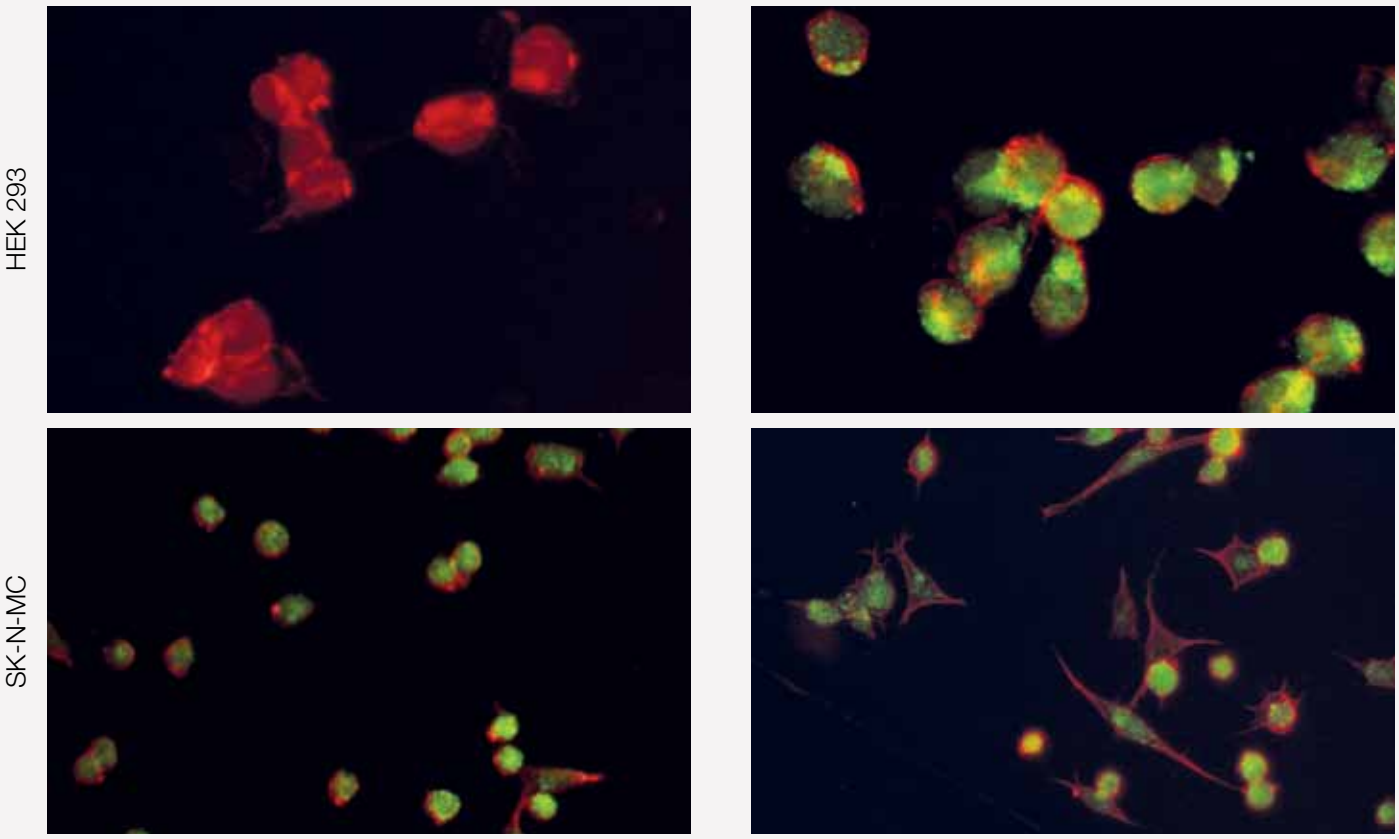


Figure 10: Staining for vinculin (green) and actin containing stress fibres (red) after 2 hours of HEK 293 (upper pictures) and SK-N-MC (lower pictures) cell attachment.

Specific formation of focal adhesion plaques were not detectable for CHO cells cultivated on the CELLSTAR® TC surface until 24 hours after attachment and vinculin staining was in general less intense when compared to Advanced TC™.
Comparable to CHO cells, serum-free cultivated HEK 293 and SK-N-MC cells displayed visible differences between the standard and the improved tissue culture surface (Fig. 10).
Due to the absence of serum proteins HEK 293 cells display a more condensed and spherical morphology especially on the CELLSTAR® TC surface. Equivalent to CHO cells, vinculin expression could only be detected when cultivated on the Advanced TC™ polymer which intensified cellular adhesion especially after washing steps and media changes (see also chapter 2.1).

Aggregations of vinculin as well as several discrete actin fibres indicate incipient formation of focal adhesion plaques in HEK 293 cells after two hours whereas a first sign of such adhesion structures could only be detected after six hours on the CELLSTAR® TC surface (data not shown).

Beside faster cell attachment and vinculin expression SK-N-MC cells exhibited a higher degree of differentiation when cultivated on the Advanced TC™ modification. Already after two hours a high number of cells with a clearly defined neuronal phenotype could be identified (Fig. 10). While expressing vinculin, SK-N-MC cells attaching to the CELLSTAR® TC surface displayed an undifferentiated morphology at this time point.

48 hours after cell seeding the proportion of differentiated SK-N-MC cells is equivalent on both growth substrates while vinculin expression is slightly intensified on the Advanced TC™ surface. Higher magnifications of these cells reveal augmented focal adhesion sites when compared to cells on the CELLSTAR® TC surface (data not shown). In summary the physical-chemical surface modification Advanced TC™ exerts a positive effect on cell adhesion and differentiation traceable by intensified vinculin expression and accumulation as well as the formation of bundled actin containing stress fibres.

2.4 Transfection

The process of introducing nucleic acids into eukaryotic cells by nonviral methods is defined as transfection. Using various chemical, lipid or physical methods, this gene transfer technology is a powerful tool to study gene function and protein expression in the context of a cell. Assay-based reporter technology, together with the availability of transfection reagents, provides the basis to study mammalian promoter and enhancer sequences, transacting proteins such as transcription factors, mRNA processing, protein-protein interactions, translation and recombination events[11].

Even though transfection is a well established laboratory process, in the majority of cases the optimal experimental settings have to be defined for each cell line and vector system. Transfection can result in unexpected morphological changes and abnormalities in target cells and some liposome-based transfection reagents can induce toxicity. The common problems researchers face in transfection are low transfection efficiency, reduced cell viability following transfection and weak transgenic expression. In general transfection performance is better when cells are seeded in the optimal densities and viable. Therefore an improved surface like Advanced TC™ can exert a positive effect on transfection experiments. Complexes which can be formed in the presence of serum reducing the amount of accessible DNA for transfection can be prevented by using serum free media. This however can negatively influence cell viability, proliferation and attachment based on the reasons discussed earlier (see chapter 1). Likewise the optimisation of a cell culture substrate like Advanced TC™ can counterbalance these effects and facilitate positive transfection results.

To analyse transfection efficiency various cell lines have been seeded in the appropriate cell density and transfected either with the plasmid pcDNA3-EGFP to express the green fluorescent protein GFP or pCMV-Gluc to express Gaussia Luciferase in mammalian cells. This Luciferase is naturally secreted from mammalian cells in an active form. Thus levels of Luciferase in the conditioned medium are linear with respect to cell number, growth and proliferation[12,13]. The enzyme catalyses the oxidation of the substrate coelenterazine in a reaction that emits light at a wavelength of 470 nm. Transfection efficiency can therefore be detected by measuring Luciferase activity in the culture supernatant via luminescence reading. For GFP expression cells can be analysed using a fluorescence microscope. By counterstaining cells with Alexa Fluor® 594 wheat germ agglutinin to visualise the plasma membrane and Hoechst 33342 to stain cell nuclei transfected cells can be separated from untransfected and enumerated (Fig. 11).

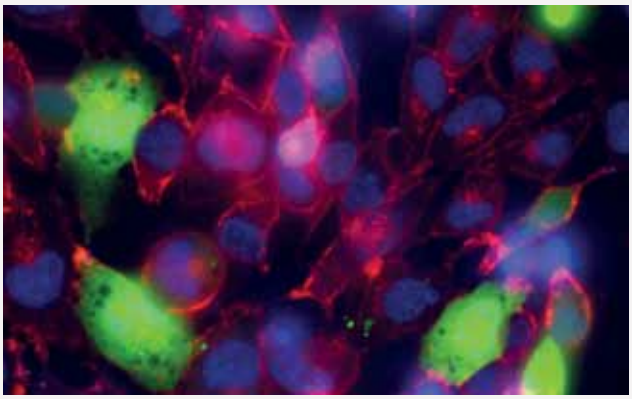


Figure 11: GFP transfection and immunocytochemistry of CHO cells with Alexa Fluor® 594 wheat germ agglutinin (red) to visualise the plasma membrane and Hoechst 33342 (blue) to stain cell nuclei.

In addition to an optical evaluation which enables morphological analysis, the overall fluorescence can be determined using a fluorescence plate reader.

For each tested cell line Advanced TC™ exerted a positive effect. Both the microscopic as well as the reader based analysis revealed a higher proportion of transfected cells or magnified fluorescence signal per well.

In correlation to the standard tissue culture surface CHO cells displayed a 33% higher fluorescent signal when cultivated on the Advanced TC™ surface whereas serum-free cultivated HEK 293 cells led to 17% and HepG2 cells to 9% increase in fluorescence (Fig. 12).

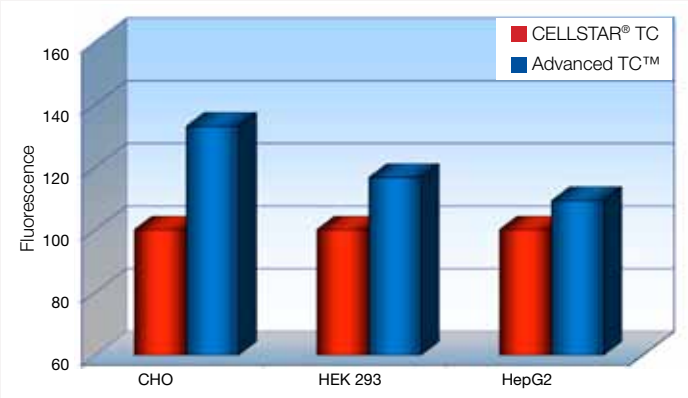


Figure 12: GFP transfection

Analogous to the GFP transfection experiment Advanced TC™ also positively influenced the Luciferase expression of CHO, HEK 293 and HepG2 cells (Fig. 13). CHO cells displayed a 44% and HepG2 an 18% higher luminescent signal. The most prominent effect was visible for serum-free cultivated HEK 293. In this case the luminescent signal was doubled to 102%.

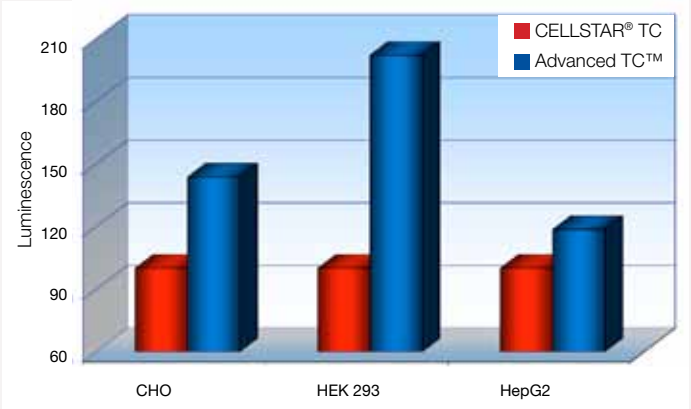


Figure 13: Luciferase transfection

Comparing the results from the GFP and the Luciferase expression it becomes clear, that transfection experiments can differ from each other based on the vector or plasmid which has to be inserted into the cell.

But independent of experimental settings, the cultivation of cells on the Advanced TC™ surface prior and during the experiment facilitates higher transfection efficiencies, better cell survival and stable gene expression after transfection.

2.5 Albumin secretion

Tissue functions depend on the reciprocal and dynamic interactions of cells with their surrounding microenvironment, which includes biochemical and mechanical stimuli defined by neighbouring cells and extracellular matrices[14].

In addition to anchoring cells, adhesive interactions activate various intracellular signalling pathways that direct cell viability, proliferation and differentiation[4,5]. The latter can be influenced by surface chemistries[7] and determined for example by albumin secretion of liver cells:

Albumin content was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) adapted from Holzmann et al[15].

Assessment of hepatic albumin secretion as a marker of liver-specific protein synthesis indicated increased hepatic function on the Advanced TC™ surfaces as compared to the CELLSTAR® TC control (Fig. 14).

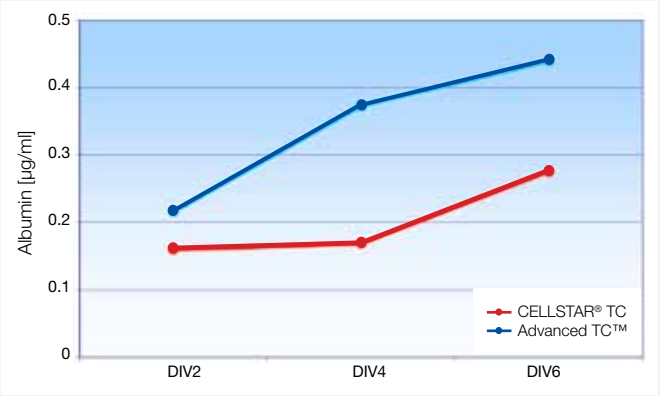


Figure 14: Albumin secretion

Previous experiments (see chapter 2.2) have illustrated the positive effect of the Advanced TC™ modification on the morphology and adherence of HepG2 cells. Faster cell attachment and optimal adhesion leads to prior induction of differentiation when compared to standard tissue culture surfaces. This is apparent already at day two *in vitro* (DIV2) with higher albumin protein levels in the supernatant. While HepG2 cells cultivated on the CELLSTAR® TC surface display higher liver specific protein synthesis by DIV4, the identical cell population secretes 82% more albumin on the same day when Advanced TC™ is used as cell substrate and 208% more on DIV6 (**Fig. 15**).

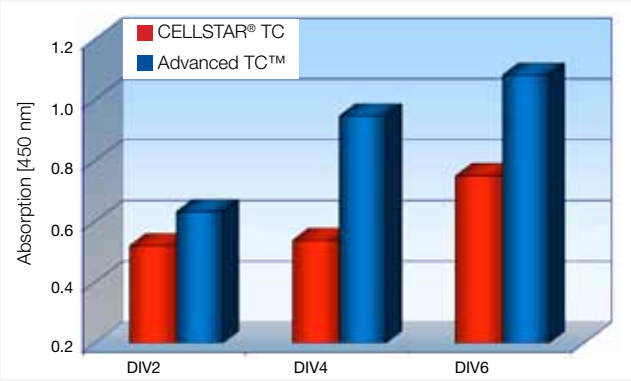


Figure 15: Albumin secretion

These results make clear that an optimal cell adherence can positively influence cell physiology, signalling pathways and differentiation.

3. Summary

There is strong interest in being able to control precisely adhesion and growth characteristics of cells in culture. The surface state of the substrate is of vital importance in the determination of its applicability in cell culture. The understanding of the relationship between cells and the physiochemical properties of the surface (such as charge, wettability, functional groups and topography) is of prime importance for the optimisation of adhesion, spreading and cell growth. It has been observed that the specific surface chemistry and polar free energy of a polymer substrate can influence the adhesion and subsequent cell growth process, either directly or indirectly through the serum protein layer which rapidly absorbs onto the surface prior to cell adhesion[7].

Consequently surface modification treatments such as plasma or corona together with the absorption of proteins and surfactants have been shown to positively influence the characteristics of the cell growth process[2].

Current standard practices for growing adherent cells in cell culture involves the use of defined chemical media to which bovine, fetal calf or other animal serum is added. This supplement provides nutrients and promotes cell adhesion by coating the treated plastic surface with a biolayer matrix to which cells can better adhere.
While advantageous for cell growth, serum can have adverse effects by introducing sources of infection or abnormally inducing expression of unwanted genes when cells are exposed to serum.
While the mechanisms for cell attachment to non protein coated substrates is not fully understood, it is believed to be based on three general characteristics; surface morphology, chemical functionalities and surface energy.

Based on this knowledge Greiner Bio-One has developed the novel Advanced TC™ surface with the aim to facilitate serum-free cultivation of cells as well as to optimise the overall performance of cells in culture. As displayed previously the novel Advanced TC™ cell culture surface improves cell adherence, leading to consistent and homogenous cell attachment, an *in vivo*-like morphology, a high degree of differentiation and minimised cellular detachment during media changes or washing steps. It facilitates the cultivation of fastidious and sensitive cells as well as usage of serum-reduced or serum-free media. The optimal cultivation conditions accelerate proliferation, increase cell yield and maximise transgen activity in transfected cells. In summary the innovative Advanced TC™ technology optimises cellular performance leading to better assay consistencies.

3.1 List of cell lines

Cells that have been successfully cultured on CELLSTAR® Advanced TC™:

Primary Cells	Brain gangilia*
	Chicken gut cells*
	Dendritic cells
	Ewing sarcoma cells
	Hepatocytes*
	Hippocampal neurons*
	Primary neuroblastoma cells
	Rhabdomyosarcoma cells
Stem Cells	Murine CGR8
	Murine embryonic ES-D3
	Murine NDC
	Murine bone narrow mesenchymal stem cells
Cell Lines	293FT ViraPower virus production cells
	3T3L1 adipose like mouse embryonic fibroblasts*
	A549 human lung carcinoma cells
	CHO chinese ovarian hamster cells
	C22 lung cells*
	HEK 293 human embryonic kidney cells
	HEK 293 human embryonic kidney cells; serum-free
	HeLa cells
	HepG2
	HepG2 21
	Human brain endothelial cells
	Human dermal blood endothelial cells
	HUVEC
	NH3T3*
	NL H441 human pulmonic endothelial cells
	PBMEC porcine brain microves-sel endhotelial cells*
	PC12
Cell Lines	Sertoli cells*
	SK-N-MC
	TaT1 cells*
	U2OS
	U87 gliablastoma cells
	UKF-NB3 neuroblastoma cells
	V143 cells; serum free
	Vero cells
Whole Organisms	Chicken embryos

*Cells displayed no visible difference to CELLSTAR® TC

List of analysed cells is permanently updated on our website. Please refer to:
www.gbo.com/bioscience/product_information

3.2 Further Information

F076 036 Application Report
Advanced TC™ – Cultivation and Differentiation of Embryonic Stem Cells, 6/2010 (English)

F076 033 Reprint Bioforum Europe
Challenging Cell Culture Experiments
11/2008 (English)

F076 032 Reprint Laborpraxis
Modifizierte Oberflächen optimieren Zellanalyse
10/2008 (German)

F076 034 Product information Advanced TC™ (English)

F076 031 Product Information Advanced TC™ (German)

You can either download the PDF files on our website: www.gbo.com/bioscience
or order a copy at your local reseller or Greiner Bio-One subsidiary.

4. Ordering Information

Cat.-No.	Description
655 980	96 Well, F-bottom/chimney well, with lid, transparent, Advanced TC™, sterile
655 982	96 Well, F-bottom/chimney well, with lid, transparent, Advanced TC™, sterile
655 983	96 Well, F-bottom/chimney well, with lid, µClear®, white, Advanced TC™, sterile
655 986	96 Well, F-bottom/chimney well, with lid, µClear®, black, Advanced TC™, sterile
675 983	96 Well, Half Area, with lid, µClear®, white, Advanced TC™, sterile
675 986	96 Well, Half Area, with lid, µClear®, black, Advanced TC™, sterile
781 983	384 Well, F-bottom, µClear®, white, with lid, Advanced TC™, sterile
781 986	384 Well, F-bottom, µClear®, black, with lid, Advanced TC™, sterile
788 983	384 Well, Small Volume™, µClear®, white, LoBase, with lid, Advanced TC™, sterile
788 986	384 Well, Small Volume™, µClear®, black, LoBase, with lid, Advanced TC™, sterile
657 960	6 Well Plate, with lid, Advanced TC™, sterile
665 980	12 Well Plate, with lid, Advanced TC™, sterile
662 960	24 Well Plate, with lid, Advanced TC™, sterile
677 980	48 Well Plate, with lid, Advanced TC™, sterile
627 960	Cell Culture Dish, Ø 35 x 10 mm, Advanced TC™, sterile
628 960	Cell Culture Dish, Ø 60 x 15 mm, Advanced TC™, sterile
633 971	Cell Culture Dish, Ø 94 x 16 mm, Advanced TC™, sterile
664 960	Cell Culture Dish, Ø 100 x 20 mm, Advanced TC™, sterile
639 960	Cell Culture Dish, Ø 145 x 20 mm, Advanced TC™, sterile
779 960	AutoFlask™, for automated systems, Advanced TC™, sterile, Barcode labelling, Colour code blue
690 975	Filter Cap Cell Culture Flask, 25 cm^2, Advanced TC™, sterile, Filter screw cap blue
658 975	Filter Cap Cell Culture Flask, 75 cm^2, Advanced TC™, sterile, Filter screw cap blue
660 975	Filter Cap Cell Culture Flask flat, 175 cm^2, Max. Volume 550 ml, Advanced TC™, sterile, Filter screw cap blue
661 975	Filter Cap Cell Culture Flask high, 175 cm^2, Max. Volume 650 ml, Advanced TC™, sterile, Filter screw cap blue
690 960	Cell Culture Flask, 25 cm^2, Advanced TC™, sterile, Standard screw cap blue
658 970	Cell Culture Flask, 75 cm^2, Advanced TC™, sterile, Standard screw cap blue
660 960	Cell Culture Flask flat, 175 cm^2, Max. Volume 550 ml, Advanced TC™, sterile, Standard screw cap blue
661 960	Cell Culture Flask high, 175 cm^2, Max. Volume 650 ml, Advanced TC™, sterile, Standard screw cap blue

5. Literature

1 Nichols, A. Methods in Molecular Biology. (2007) 356: p. 379-387

2 Teare D. Langmuir. (2000) 16: p. 2818-2824

3 Cooke M.J. Cytotechnology (2008) 56: p. 71-79

4 Hynes R.O. Cell (2002) 110: p. 673-687

5 Giancotti F.G. Science (1999) 285: p. 1028-1032

6 Keselowsky B.G. Biomaterials (2004) 25: p. 5947-5954

7 Ni M. International Journal of Molecular Sciences (2009) 10: p.5411-5441

8 Keselowsky B.G. PNAS (2005) 102: p. 5953-5957

9 Brakebusch, C. EMBO Journal (2003) 22: p. 2324-2333

10 Murphy-Ullrich J. The Journal of Clinical Investigation (2001) 107: p. 785-790

11 Groskreutz and Schenborn. Methods of Molecular Biology (1997) 63: p. 11-30

12 Badr C.E. PLoS One (2007) 2: e571

13 Tannous B.A. Molecular Therapie. (2005) 11: p. 435-443

14 Chen A. Biomaterials. (2009) 30: p. 1113-1120

15 Holzman MD. Transplantation. (1993) 55: p. 1213-1219

Revision 04/2011 - F071 104

Germany (Main office): Greiner Bio-One GmbH, info@de.gbo.com I **Austria:** Greiner Bio-One GmbH, office@at.gbo.com
Belgium: Greiner Bio-One BVBA/SPRL, info@be.gbo.com I **Brazil:** Greiner Bio-One Brasil, office@br.gbo.com
China: Greiner Bio-One GmbH, info@cn.gbo.com I **France:** Greiner Bio-One SAS, infos@fr.gbo.com
Japan: Greiner Bio-One Co. Ltd., info@jp.gbo.com I **Netherlands:** Greiner Bio-One B.V., info@nl.gbo.com
UK: Greiner Bio-One Ltd., info@uk.gbo.com I **USA:** Greiner Bio-One North America Inc., info@us.gbo.com

greiner bio-one