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Challenging Cell Culture Experiments

Improve Your Results with an Innovative Surface Optimising Cell Attachment, Proliferation and Transgene Activity

The innovative Advanced TC polymer modification increases cellular primary and long term adhesion alters the cell culture surface in such a way to positively influence cellular features and functions. Higher proliferation rates improve cell expansion and cultivation of sensitive cells or cells under restricted growth conditions. Due to the reproducibility of the process the novel modification of the polymer assures constant product quality.

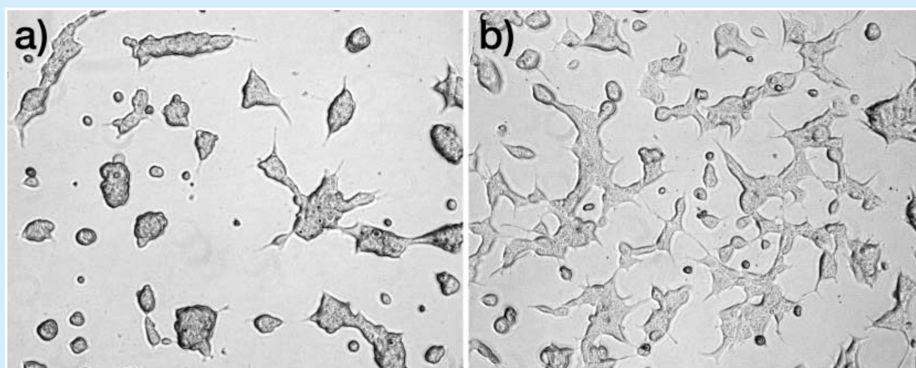

Fig. 1: Improved adherence of HEK 293 cells cultivated in serum free media
HEK 293 cells were seeded in a 96well plate with a concentration of 20,000 cells/well and cultivated in serum free media at 37°C and 5 %CO_2. After 48 hours cells are semi adherent on the standard tissue culture surface (a) whereas on the Advanced TC surface (b) HEK 293 cells display improved attachment and their cell specific morphology.

Introduction

Biotechnology is often used to refer to the genetic engineering technology of the 21st century. Combining disciplines like genetics, molecular biology, biochemistry, embryology and cell biology, biotechnology can also be defined as an all-encompassing life science. Bridging these techniques initiated the development of a variety of new applications as well as the commercialisation of modern biotechnology.

Focussing on cellular screening approaches using immortalised cell lines, primary cells or co culture models, cell based assays are nowadays one of the most important biotechnological techniques. Researchers in both the academic and industrial markets 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]. Therefore the utilisation of living cells leads to more comprehensive data, which complement results from former biochemical assays.

Obstacles of *in vitro* Cell Culture

Propagation and preservation of cells and tissue *in vitro* in general can be very 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].

On the other side cultivation of cells *in vitro* mainly refers to a two-dimensional culture on plastic surfaces lacking 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 expansion. Contemporaneous the comprised serum proteins are linked to the plastic surface creating an ECM-like surface which promotes cellular adhesion.

Due to possible variations of serum composition as well as the potential contamination risk based on the biological origin of the FCS pharmaceutical and biotechnological industries favour a serum-reduced or serum-free cultivation. These restricted growth conditions however minimise protein binding to the cultivation surface and primarily cellular adhesion. Furthermore absence of growth factors results in decelerated proliferation rates.

Beside immortalised cell lines another class of cells has gained importance for biotechnological research in the last couple of years. Primary cells explanted from the respective tissue react in a more native way when compared to estab-

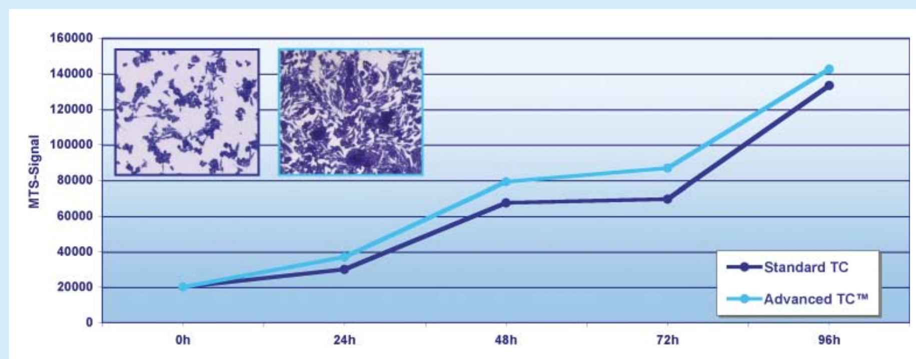

Fig. 2: Increased proliferation of SKNMC cells
SKNMC cells were seeded well in a 96well plate with a concentration of 20,000 cells/well and cultivated at 37°C and 5 %CO_2. At each indicated time point the cell proliferation rate was determined analysing cellular metabolic activity (MTS signal). Cells were additionally stained with crystal violet to identify living cells. Due to the increased proliferation rate higher cells numbers can be detected on the Advanced TC surface (light blue frame) when compared to Standard TC surface (dark blue frame).

lished immortalised cell lines. Mimicking the organ specific reaction these systems are privileged by researchers but contrariwise difficult in their cultivation. The need of an *in vivo*-like surrounding and the limited life span requires the adaption of the media composition or the respective cultivation surface. Comparable to primary cells also Co-culture models facilitate insight into complex coherences and reactions of 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 fulfilled. Preserving *in vitro* the cellular interconnection and function during the cultivation procedure is a demanding approach.

Researchers not only face drawbacks due to characteristics of the corresponding 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. Concurrently the use of robotics often results in increased cell stress or cell loss during automated washing steps and media changes. Irregular cellular attachment depending on common variances of cell culture can lead to a minor assay consistency and higher variances between individual assays. This minimises the significance or informative value of the performed experiment.

Summarising the preceding biotechnological developments as well as the cell biological requirements leads to an apparent need for the optimisation of the cultivation platforms.

Optimising Cellular Adhesion

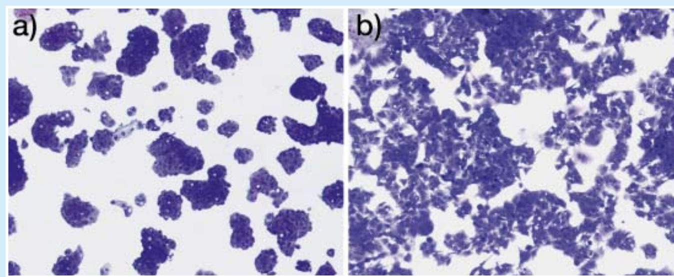

Fig. 3: *In vivo* like morphology of HepG2 cells
HepG2 cells were seeded in a Standard TC (a) or Advanced TC (b) 96well plate with a concentration of 20,000 cells/well, cultivated under identical conditions for 48 hours and stained with crystal violet. On the Advanced TC surface (B) cells display their *in vivo* like morphology.

One approach to optimise cellular primary and long term adhesion is to coat matrix specific proteins to the plastic surfaces simulating an ECM or *in vivo*-like surrounding to the cell. Poly-D-Lysine (PDL), Poly-L-Lysine (PLL), Fibronectin, Laminin as well as Collagen are the commonly used proteins for these types of coatings.

An entirely new development is the imitation of the cellular surrounding in a non-biological way. Based on this approach 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 endangered from degradation and stable for several years. Likewise the possibility of cross reaction or contamination based on a biological protein can be circumvented. Beside these concomitant product features the Advanced TC technology has a preeminent effect on cell cultivation processes:

Advanced TC facilitates in general 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 (fig. 1) and higher proliferation rates (fig. 2) improve and accelerate cell expansion, facilitating in particular cultivation of the above mentioned 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 the native *in vivo*-like morphology (fig. 3).

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 mentioned gene transfer (fig. 4).

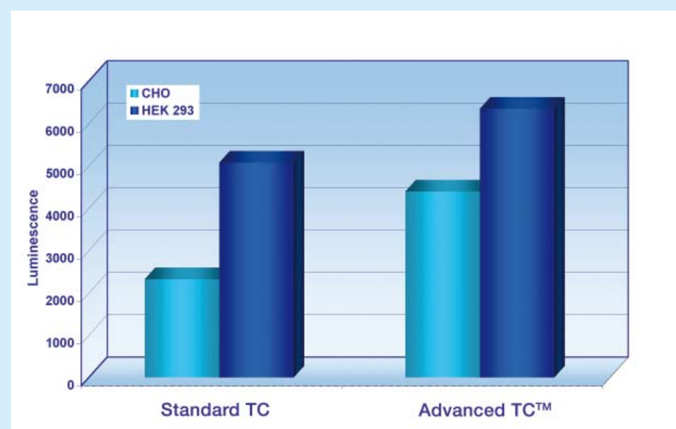

Fig. 4: Higher transgene activity of CHO and HEK 293 cells
CHO and HEK cells were seeded in a 96well plate with a concentration of 40,000 cells/well or 100.000 cells/well respectively, cultivated at 37°C and 5 %CO_2 for 24 hours and thereafter transfected with the pCMV- GLuc-vector. Both cell lines exhibit higher Luciferase activity on the Advanced TC surface.

Conclusion

The novel Advanced TC cell culture surface improves cell adherence, leading to consistent and homogenous cell attachment, an *in vivo*-like morphology 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 transgene activity in transfected cells. In summary the innovative Advanced TC technology optimises assay consistency of cell-based biotechnological screening approaches.

References

[1] Nichols A.: Methods in Molecular Biology 356, 379–387 (2007)
[2] Bacakova L. *et al.*: Physiological Research 53, S35–45 (2004)
[3] Zamir E. *et al.*: Journal of cell science 114, 3583–3590 (2001)

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