ADVANCED TC: A CELL CULTURE SURFACE IMPROVING THE CULTIVATION AND DIFFERENTIATION OF EMBRYONIC STEM CELLS

1/ INTRODUCTION

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation *in vitro*[1, 2].

The importance of embryonic stem cells rests in their lack of specialisation. These basic cells are present in the earliest stages of developing embryos and are able to develop into virtually any type of cell and tissue in the body. As such, an understanding of their unique attributes and control can lead to in-depth knowledge about early human development. Furthermore embryonic stem cells can offer a prospective limitless source of cells and tissue due to their self-renewing potential. Based on this feature embryonic stem cells have gained enormous importance over the past decades in medical science.

Purposive cell therapeutic approaches for example aim to replace damaged or diseased cells and tissues by embryonic stem cells. But due to limited knowledge in stem cell research with the first human embryonic stem cells being isolated approximately twenty years ago[3] there is still an extensive need for basic scientific investigation to understand the complexity of human diseases as well as stem cell maintenance and differentiation.

2/ CULTIVATION OF EMBRYONIC STEM CELLS

In general propagation of cells and tissue *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[4, 5].

Moreover, cultivation of cells *in vitro* mainly refers to a two-dimensional culture on plastic surfaces lacking the vital signals provided by the connective tissue. Stem cells being a very sensitive cellular system with a high risk of spontaneous differentiation under inappropriate culture conditions are therefore usually co-cultured with "feeder" cells derived from mouse or human. These feeder cells provide secreted factors such as leukemia inhibitory factor (LIF), extracellular matrix, and cellular contacts for the maintenance of stem cells in the undifferentiated state without losing their pluripotency. However feeder cells may poses a potential risk of cross-contamination such as passing animal pathogens or retroviruses to human embryonic stem cells hindering clinical application of these cells.

Although feeder-free systems[6-7] have been reported in the last couple of years, these approaches required addition of feeder conditioned medium carrying a similar risk of pathogen contamination as well as batch to batch variations.

The major aim therefore is to develop chemically defined media and conditions to eliminate the risk of infection from animal components as well as to reduce lot-to-lot variability resulting in consistent and comparable cellular behaviour and facilitating eventual use in clinical applications.

3/ FEEDER-FREE EXPANSION OF EMBRYONIC STEM CELLS ON THE ADVANCED TC SURFACE

Beside optimal chemical conditions feeder-free cultivated stem cells also require an appropriate physical environment to enable cellular survival, attachment and proliferation. To exclude any risk of cross-contamination and pathogen carryover Greiner Bio-One GmbH has developed the Advanced TC surface, a non-biological polymer modification mimicking the cellular surrounding to positively influence cell adhesion.

Cultivating embryonic stem cells on the Advanced TC surface leads to equivalent results when compared to expansion on feeder layers. Their undifferentiated state can be characterised by high levels of alkaline phosphatase **(Figure 1)** as well as the expression of the surface marker SSEA-1 and the transcription factor Oct-4 **(Figure 2)**.

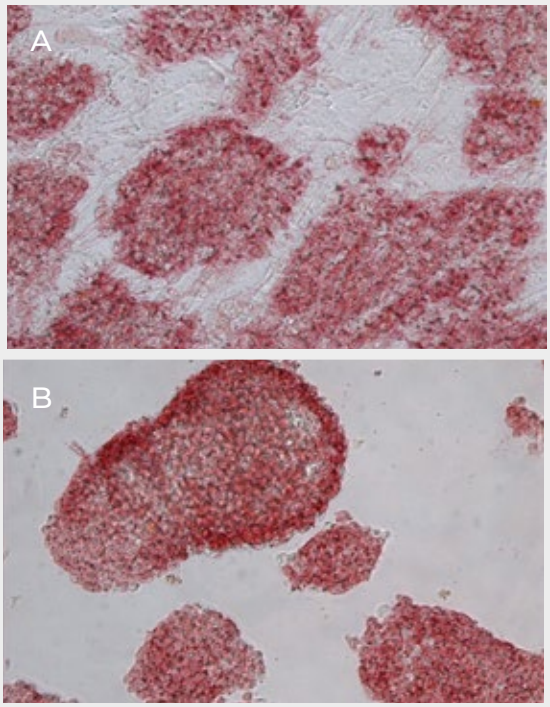

Figure 1: The embryonic stem cell line ES-D3 has been cultivated either on STO-feeder cells **(A)** or on Advanced TC surface **(B)** in LIF containing stem cell media. 4 days after seeding ES cells in 96 well plates (20,000 cells/well) alkaline phosphatase expression has been determined using the alkaline phosphatase substrate kit (Vector® Red). Pluripotent, undifferentiated stem cells are characterised by a strong red staining.

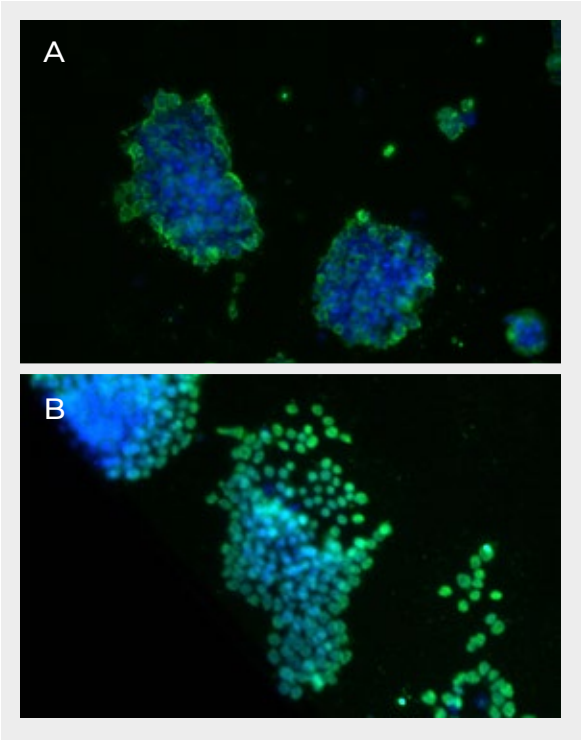

Figure 2: The ES-D3 cell line has been cultivated on the Advanced TC surface in LIF containing stem cell media. 4 days after seeding the expression of the surface marker SSEA-1 **(A)** and the transcription factor Oct-4 **(B)** has been analysed using a mouse-anti-SSEA-1 or rabbit-anti-Oct-4 antibody detected by the respective Alexa 488 coupled secondary antibody and a DAPI counter stain.

4/ DIFFERENTIATION OF EMBRYONIC STEM CELLS

Aside from the propagation of embryonic stem cells, keeping their pluripotency throughout the cultivation process, the differentiation of these cells is of major importance for their therapeutic application. One of the most promising application fields for such a cell therapy approach are neurological disorders like parkinson, stroke and multiple sclerosis. Under normal circumstances, the nervous system is incapable of healing itself.

In the case of disease or injury, patients can be left with impaired motor function, paralysis or other disorders. Stem cells, however, can be used to create new neurological cells and tissue, and in theory could be used to repair damaged cells and restore normal function.

Similar to the general cultivation of stem cells the cellular or physical environment is also critical for the differentiation process of these cells.

Induction of neuronal differentiation of the embryonic stem cell line ES-D3 cultivated on the Advanced TC surface by the addition of retinoic acid leads to a high number of neurons and facilitates long term cultivation for more then ten days after initiation of neuronal transition **(Figure 3).**

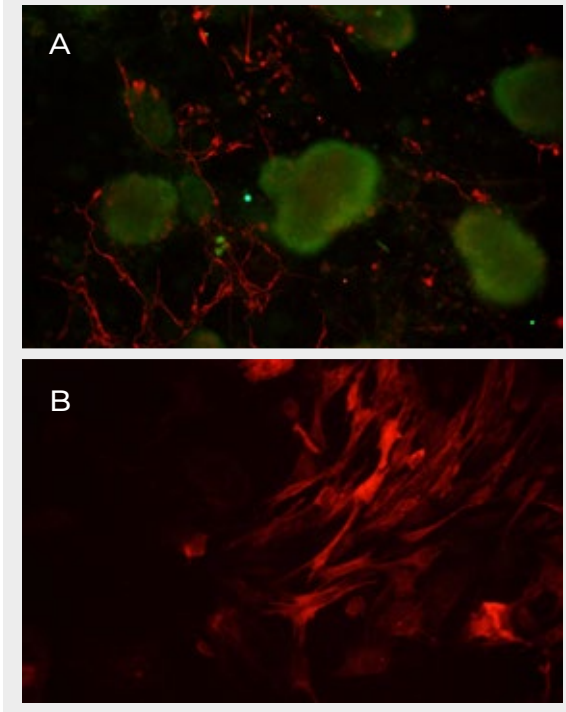

Figure 3: Neuronal differentiation of the embryonic stem cell line ES-D3 cultivated on the Advanced TC surface has been induced by the addition of retinoic acid. Expression of the neuronal specific marker ßIII-tubulin (red) and the marker for neuronal precursor cells Nestin (green) was analysed 6 **(A)** and 10 days **(B)** after induction of neuronal differentiation. While after 6 days neuronal precursor cells are still detectable, a pure neuronal culture can be reached within 10 days.

5/ CONCLUSION

The Advanced TC cell culture surface improves cell adherence, leading to optimal condition for embryonic stem cell cultivation while excluding any risk of cross-contamination and pathogen spreading possibly caused by feeder cells. Embryonic stem cells remain pluripotent determined by the high expression lever of alkaline phosphatase, the surface marker SSEA-1 and the transcription factor Oct-4.

Beside the positive effect during the general propagation of these cells Advanced TC also supports neuronal differentiation by retinoic acid induction and long term cultivation of neuronal cells. In summary these results emphasise the capability of Advanced TC as a powerful tool for embryonic stem cell research and encourage new therapeutic approaches.

6/ LITERATURE

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