Introduction

- Embryonic stem cells (ESCs) have gained considerable interest in recent years due to their capacity to both self-renew and differentiate into cells of all three germ layers.
- They can develop into any type of cell or tissue in the body and are especially attractive for regenerative therapies.
- Widespread use of ESCs for therapeutic approaches has been hampered by the complexity of the conditions required for their maintenance and differentiation. Therefore scientists try to establish protocols which reliably direct the differentiation of embryonic stem cells into specific cell types.

In the presented set of experiments, we show that murine embryonic stem cells can be cultivated on Greiner Bio-One surfaces keeping them in an undifferentiated state. Furthermore, we present a simple though efficient protocol for the differentiation of ESCs into neurons.

Undifferentiated Embryonic Stem Cells on Advanced TC™ without Feeder Cells

- Maintenance of undifferentiated ESCs is necessary with controlled conditions such as
  - Fibroblast feeder layer
  - Supplemental leukemia inhibitory factor (LIF).
- Test for differentiation status of cells is done with cellular markers such as alkaline phosphatase (AP) stage-specific antigen-1 (SSEA-1) or transcription factor Oct-4.
- Culture of mouse ES-D3 embryonic stem cells on Greiner Bio-One CELLSTAR® TC and Advanced TC™ cell culture products in LIF-containing medium allows keeping the cells in an undifferentiated state for several days without feeder cells (Fig. 1, 2).

Figure 1: Alkaline phosphatase staining of murine ES-D3 embryonic stem cells cultivated for four days on inactivated STO cells (left) or on the indicated Greiner Bio-One surfaces (right, magnification 20x). Red staining indicates high alkaline phosphatase expression typically seen with undifferentiated stem cells.

Neuronal Differentiation of Embryonic Stem Cells on Advanced TC™ surface

- Neuronal differentiation of ESCs can be achieved by the addition of retinoic acid (RA).
- The antimetabolic agent cytosine arabinoside (CAR) inhibits proliferation of contaminating, rapidly dividing, non-neuronal cells.
- Neuronal differentiation can be examined by expression of nestin (early neuronal differentiation), βIII tubulin (neurons) and GFAP (glial fibrillary acidic protein), non-neuronal glia cells) with immunocytochemistry.
- High number of neurons on CELLSTAR® TC and Advanced TC™ surface in neuronal differentiation experiment, indicated by nestin and βIII tubulin expression (Fig. 4).
- More differentiated neurons detected when CAR was added.
- Addition of CAR minimises GFAP-positive non-neuronal cells, shown also by the absence of morphologically defined glial cells (Fig. 5).
- High number of neuronal interconnections (= highest neuronal differentiation) with Advanced TC™ polymer modification.
- Optimal neuronal differentiation of murine ESCs is achieved with addition of CAR and culture on Advanced TC™ surface.

Conclusion

- Adherent embryonic stem cells can be effectively cultivated and expanded on Greiner Bio-One surfaces like CELLSTAR® TC and Advanced TC™.
- Embryonic stem cells remain pluripotent without feeder cells as determined by their high expression of alkaline phosphatase, SSEA-1 and Oct-4.
- Defined experimental conditions with usage of Advanced TC™ as no non-embryonic cells can interfere with e.g. staining results.
- Very effective and direct neuronal differentiation could be achieved by the addition of retinoic acid and selection with cytisine arabinoside on the Advanced TC™ polymer modification without the need of embryoid body formation.
- In summary, these results emphasise the capability of Advanced TC™ as a powerful tool for embryonic stem cell research facilitating cultivation and neuronal differentiation of murine embryonic stem cells on a non-biological, xeno-free surface.

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Improved Cultivation and Differentiation of Embryonic Stem Cells


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