Potential therapeutic applications of embryonic stem cell (ESC)-derived hepatocytes as an alternative to the transplantation of the whole liver or of primary hepatocytes is currently confirmed to be a highly topic issue. Crucial points thereby are high-yield generation and efficient selection of cells displaying hepatocyte-specific features from differentiating ESC cultures. In our study, we establish culture conditions for a large-scale production of hepatocyte-like cells from ESCs in a murine in-vitro model, using stable transgenic ESC clones which contain the live eGFP reporter gene and a puromycin resistance cassette, both driven by a common alpha-fetoprotein (AFP) gene promoter. From that clones, suspension- and adherent cultures with the activated AFP promoter were generated, which derived from the endoderm-like cell population. These cultures are supposed to express eGFP fluorescence as well as to acquire puromycin resistance, thus allowing for live monitoring of both differentiation and antibiotic selection.

We found out that a 95%-yield of eGFP-expressing embryoid bodies (EBs) can be achieved by using a spinner flask suspension culture. This culture seems to be temporally synchronised in terms of the rate of eGFP-positive cells and of their localization pattern in the outer rim of EBs. Features of cells selected from the EB culture by puromycin application were shown to be dependent on the time point of the initial drug application. Thus, the puromycin treatment of EBs on their relatively early developmental stage yielded highly eGFP-expressing cell clusters that, after their plating onto adhesive substrates, developed to a culture comprising both highly proliferative hepatocyte-precursor-like- and advanced differentiated hepatocyte-like cells. Exposure of a long-term adherent culture to the drug resulted rather in selection of a significantly more mature cell population displaying a low proliferation capacity. We also investigate the effect of different adhesive substrates on the developmental pattern of adherent cultures.

Hence, alteration of culture conditions allows for differentiation of murine ESCs toward cells of the hepatic lineage and their selection on defined developmental stages, which is of interest in regard to toxicology screening- and transplantation models.