Expansion of Mouse Embryonic Stem Cells in Suspension Culture Using CultiFlask 50 Tubes
Magda Tomala¹, Pierre Moretti¹, Cornelia Kasper¹, Ulrich Martin², and Thomas Scheper¹
Corresponding Author: kasper@ifc.uni-hannover.de
¹Institute of Technical Chemistry, Leibniz University of Hannover, Germany
²Department of Cardiac-, Thoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Germany

Introduction
Embryonic Stem (ES) cells are self-renewing and pluripotent cells with the ability to differentiate into a variety of cell lineages. Implementation in tissue engineering or as model systems for drug discovery makes ES cells attractive as a cell source. To use ES cells for these applications, technologies are required to generate a large number of cells with defined characteristics. Dynamic bioreactor cultivations enable a homogenous environment and facilitate the ability to monitor and control culture parameters (e.g., oxygen content and pH) which is advantageous compared with static culture vessels. The aim of this work was to characterize CultiFlask® 50 (Sartorius Stedim-Biotech) tubes for an expansion of undifferentiated ES cells in long term suspension culture.

Materials and Methods
CultiFlask® 50 tubes containing 15 ml ES cell medium were inoculated with 2 x 10⁴ cells / ml each and shaken orbitally at an agitation rate of 270 rpm. The cultures were monitored daily by cell number determination and measurement of glucose and lactate concentrations. Maintenance of pluripotency was evaluated by expression analysis of the pluripotency markers Oct-3/4 and SSEA-1 via flow cytometry. Subsequently, ES cell spheres were induced to spontaneous differentiation by the formation of Embryoid Bodies and were analysed by RT-PCR.

Results
Figure 1 shows proliferation and viability of the cells cultivated in CultiFlask® 50 tubes. Brachyury ES cells proliferated well in CultiFlask® 50 tubes throughout six continuous passages and remained high viability values. After the expansion phase pluripotency marker expression was analysed via flow cytometry revealing high expression levels of SSEA-1 (98%) and Oct-3/4 (91.4%). Analysis of the gene expression profile showed that the cells were able to differentiate into cell types of all three germ layers.

Discussion and Conclusions
This study demonstrates the successful propagation of mouse ES cells in their undifferentiated state under suspension conditions using CultiFlask® 50 tubes. This study may contribute to the development of scalable bioreactor cultivations of embryonic stem cells.

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Disclosures
The authors have declared no conflict of interest.