Preparation of Microvascular Endothelial Cells for Cultivation with Embryonic Stem Cell-Derived Cardiomyocytes
S. Schmitteckert1, C. Ziegler1, M.X. Doss2, J. Hescheler2, A. Sachinidis2, A. Rolletschek1
stefanie.schmitteckert@kit.edu
1Institute for Biological Interfaces, KIT Campus North, P.O.B. 3640, D-76021 Karlsruhe
2Center of Physiology and Pathophysiology, Institute of Neurophysiology, Universität Köln, Robert Koch Strasse, D-50931 Köln

Introduction
Differentiation experiments using embryonic stem (ES) cells are traditionally performed in conventional tissue culture plates by adding soluble growth factors and extra-cellular matrix (ECM) components to the medium. However, this approach does not consider the three-dimensional (3D) organization of in vivo differentiating cells and the influence of a cell specific ECM composition. For engineering mature and long-term functional tissues in vitro, sophisticated cultivation systems are required that mimic the spatiotemporal regulation of tissues developing in vivo. It is well known that endothelial cells in the heart exhibit the property of close anatomical and functional interaction with cardiomyocytes. They express and release a variety of auto- and paracrine agents (e.g. endothelins) as well as extracellular matrix proteins (fibronectin, collagen IV, laminin) which directly influence cardiac development and function. Therefore, we want to establish a co-cultivation system of mouse ES cell-derived cardiomyocytes and freshly isolated microvascular endothelial cells (MVECs). Our studies will focus on the influence of primary microvascular endothelial cells on the differentiation and maturation of ES cell-derived cardiomyocytes.

Materials and Methods
For the generation of ES cell-derived cardiomyocytes we used a transgenic ES cell line exhibiting puromycin resistance and expressing eGFP under the control of the alpha-myosin heavy chain (MHC) promoter (1). ES cells were cultured as embryoid bodies (EBs). During EB formation eGFP fluorescence increased and was microscopically detectable after 8 to 10 d. During following puromycin treatment puromycin-resistant eGFP-expressing alpha-MHC+ cells within beating clusters were progressively enriched. MVECs were isolated from hearts of E18.5 mouse fetuses. The obtained unpurified cell mixture consisted predominantly of MVECs. In order to optimize the co-culture conditions different media compositions were tested. Therefore, both cell populations were analyzed by RT-PCR and immunocytochemistry (ICC) at various time points.

Results
The RT-PCR analyses with alpha-MHC-eGFP+ cells and the isolated unpurified endothelial cell population at various time points revealed no significant differences at the transcript level for e.g. Cx43, Ednra, PreproEdn1 or Edn1 comparing the ES cell differentiation medium and the mixed medium (differentiation medium and MVEC cultivation medium, 1:1). ICC of alpha-MHC-eGFP+ cells and the isolated endothelial cells for cell type specific markers revealed no significant differences between the media variants confirming the RT-PCR results. Furthermore, we performed ICC with alpha-MHC-eGFP+ 7d EB-derived cardiomyocytes and the isolated unpurified endothelial cell population cultivated in mixed medium for 7d.

Discussion and Conclusions
Results of RT-PCR analyses and ICC suggested that the use of mixed medium conditions is suitable for further co-cultivation experiments. Preliminary data of co-cultivation of 7d EBs and isolated unpurified endothelial cell population indicated an advanced and pronounced induction of cardiac differentiation of ES cells. Our future studies will focus on the influence of the organ specific MVECs on the further ES cell differentiation into mature cardiac phenotypes.

References