TOWARDS BIOHYBRID LUNG DEVELOPMENT: ANALYSIS OF CLINICALLY RELEVANT WORKING CONDITIONS ON THE ENDOTHELIAL CELLS ON GAS EXCHANGE MEMBRANES

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Introduction

The development of the implantable biohybrid lung (BHL) is intended to offer an alternative to lung transplantation, which is currently the only treatment option for patients with end-stage lung disease. Based on the same functional principle as extracorporeal membrane oxygenation (ECMO), gas exchange in the BHL is achieved by diffusion via the gas exchange hollow fiber membranes (HFM). However, these are covered with a layer of endothelial cells to prevent obstruction due to blood clotting and thrombus formation, which is normally caused by the inevitable blood contact with the artificial surfaces of conventional ECMOs. In future BHL, endothelial cells must withstand a supraphysiological high oxygen gradient between the oxygen-rich gas in the HFM and the hypercapnic/hypoxic patient blood. Thus, we investigated the influence of this clinically relevant oxygen tension under static and dynamic conditions on the endothelial cells.

Methods

Prior to seeding with 1.52 x 10^5 EC/cm², hydrophobic PMP membranes (foil for static experiments and HFM for flow exposure) were coated with 2 µg/cm² fibronectin to facilitate EC adhesion [2, 3]. After reaching confluence, ECs were pre-cultured for 24 hours under blood gas levels of severe respiratory insufficiency (50 mmHg pO_2, 80 mmHg pCO_2) and then exposed to hyperoxia (>95% pO_2) for 24 hours. In a custom-built miniature oxygenator, the endothelialised HFM were gently adapted to the flow conditions by applying an increasing ramping flow rate profile, starting at 2 ml/min and doubling every 30 minutes up to a maximum of 15 ml/min.

Remaining ECs were detected with the nuclear dye Hoechst 33342 and the vital dye calcein. Immunostaining agents were applied for the detection of extracellular matrix Collagen type-IV and cell junction protein VE-Cadherin via confocal laser scanning microscopy (CLSM). Expression level change of oxidative stress (HMOX1, GCLM) - or inflammation (ELAM, VCAM, ICAM) related genes were measured via qRT-PCR. Apoptosis (AnnexinV/PJ) and reactive oxygen species (ROS) accumulation (CellRox) were investigated using flow cytometry, and compared to menadione (50 µM, 4 h) treated ECs as positive control.

Results

On film and HFM samples, CLSM imaging confirmed confluent and viable EC-monolayers with intercellular junctions and *de novo* synthesized Collagen-IV under pre-cultivation or hyperoxia condition. In addition, no change in prothrombotic and proinflammatory gene regulation was detected. Under hyperoxia, genes associated with oxidative and flow stress were correspondingly upregulated.

The proportion of apoptotic ECs was not significantly increased in response to the high oxygen tension, while the level of ROS production was elevated. However, the ROS level of menadione-treated ECs, used as positive control, exceeded this level significantly.

Discussion

We have shown that the high oxygen tension, prevailing in the future BHL can be tolerated by the membrane seeded ECs, for at least 24h. The ECs responded to these clinically relevant conditions with an upregulation of genes related to stress and ROS-coping mechanisms, which may explain the fact that no lethal damage of the ECs could be detected.

Thus, the results of this study underline the feasibility of the biohybrid lung application under clinical conditions.

References

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