Patient-specific iPS cells for endothelialisation of membrane surfaces of implantable biohybrid lung devices

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Limited durability of lung assist devices due to clotting and thrombus formation on blood contacting surfaces is a major hurdle on the way to long-term usable biohybrid lungs. Direct contact of the polymeric materials to the blood flow results in activation of the coagulation cascade and acute thrombus formation. To reduce thrombocyte adhesion anti-thrombogenic, inert membrane materials such as poly 4-methyl-1-pentene were used, and non-covalent surface coatings like heparin, were applied. Although heparin coating improves the hemocompatibility of the membrane materials, it can so far not fully provide the antithrombogenic properties of an intact endothelial cell layer, and long term functionality could not be achieved yet. Therefore, stable endothelialization of blood contacting materials in a membrane oxygenator has been proposed as alternative approach to generate a non-thrombogenic surface. Besides neonatal human umbilical vein endothelial cells (HUVECs), EPCs as well as primary ECs isolated from saphenous veins, ECs derived from human pluripotent stem cells might represent an autologous cell source for seeding of membranes to improve hemocompatibility. The contemplable cell sources are compared concerning their expansion potential, and in particular after extended culture expansion that would be necessary to produces the required high cell numbers, genomic stability, gene expression pattern and cytokine profiles are comparatively investigated. First results concerning proliferation potential as well as genomic stability suggest that hPSC derived ECs might represent an well expandable, more stable cell source for endothelialization than neonatal or adult primary ECs. In addition following seeding onto fibronectin-coated PMP films, the hPSC derived ECs were analyzed for relevant phenotypic and functional properties under static and flow conditions. The hPSC-ECs were able to populate the PMP membranes, while preserving their major functional properties. Specifically, WST 8 and nuclei counting revealed that the cells maintained their proliferation capacity. Following confluency, the activation, thrombogenic and inflammation status of the ECs was maintained, as evidenced by gene expression analysis and leucocyte attachment testing. Moreover, the integrity of established monolayers was shown by VE-Cadherin staining, and their self-healing capacity was visualized by scratch assays. In addition, the monolayers were able to withstand arterial-equivalent flow for 24hrs, while maintaining their major gene expression characteristics. Subsequently, hiPSC-ECs were seeded onto PMP hollow-fiber membranes, where they were able to attach and form a confluent monolayer, as evidenced by VE-cadherin staining.

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Biohybrid lungs endothelialized with allogeneic MHC-silenced endothelial cells to prevent transplant rejection

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For development of biohybrid lungs as alternative to lung transplantation, poly-4-mehtly-1-pentene gas exchange membranes (PMP) will be endothelialized with allogeneic human cord blood derived endothelial cells (HCBEC) due to insufficient autologous cell harvesting. To prevent immune rejection of HCBECs due to disparities at the Major Histocompatibility Complex (MHC) loci, MHC expression will be permanently silenced using RNAi technology and lentiviral vector delivery of the shRNA. HCBECs capacity and functionality required for endothelialisation of PMP will be investigated.

MHC class I molecules of HCBEC were silenced using a lentiviral vector encoding for shRNAs targeting ß2-microglobulin. As control a non-specific shRNA was used. Efficiency of MHC-silencing was analyzed by FACS and endothelial phenotype (e.g. CD31) by RT-PCR. Relative expression levels of endothelial specific activation (e.g. ICAM, VCAM) and thrombogenic state markers (e.g. Tissue factor, Thrombomodulin) were quantified using real-time RT-PCR. TNFα-stimulation was used to examine their biologic reactivity. Cytokine secretion profile was characterized using Luminex technology. Cell growth and seeding efficiency were verified by fluorescence and scanning-electron microscopy.

HCBEC silencing showed an efficiency of 85% with a stable endothelial phenotype. Relative expression levels of endothelial specific activation and thrombogenic state makrers were unaffected by MHC-silencing. However, those levels could be induced by TNFα-stimulation showing the capacity of MHC-silenced HCBECs to react to external stimuli. The functionality of MHC-silenced HCBECs was also shown by their capacity to secrete IL-1ß and IL-8 after TNF-α stimulation. Microsopic analysis showed a nearly confluent endothelial monolayer on PMP demonstrating their capacity to fully endothelialize PMP membranes.

These results may represent a new therapeutic concept in field of organ transplantation and may bring the bioartifical lung as alternative of lung transplantation for patients with end-stage lung diseases closer to reality.

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Towards an Implantable Lung – A Surgeon´s View

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Extracorporeal membrane oxygenation (ECMO) is an established therapeutic tool in modern intensive care units, which is employed in three main conditions, including hybercarbic ventilatory failure, hypoxaemic respiratory failure and cardiac/cardiopulmonary failure. Current ECMO strategies vary among these conditions, but all of the currently clinically established units share the following common features:

- Percutaneous vascular access connected with tubing to the ECMO unit,
- A pump to maintain blood flow through the ECMO unit, coupled with a control panel for continuous measurement and regulation of blood flow,
- A gas exchange unit connected to an oxygen/gas supply system and
- A heating system to prevent blood cooling and hypothermia.

The most frequent complication associated with the use of ECMO is the risk of thrombosis and associated thromboembolism, arising from the direct contact of the blood and the artificial materials comprising the ECMO components, necessitating anticoagulation. Another potential complication is bleeding resulting from anticoagulation regime.

These challenges limit ECMO usage to application in specialized ICU settings as a short term support. With further technical improvement, ECMO support can be successfully applied in a longer-term setting and could be extended to months or even years as a bridge to transplantation, or as a potential alternative to lung transplantation. In order for this to become a realistic option, ECMO devices must become partly or fully implantable, analogous to currently available left ventricular assist devices (LVADs), which successively progressed from bulky units to fully implantable devices over the last decade.

In this project, we propose the evolution of current ECMO strategies towards implantable devices, with the aim of allowing pulmonary and/or cardiopulmonary support for longer-term periods in an outpatient setting. To achieve this, ECMO devices need to be miniaturised to become easily implantable, safe and effective. This involves overcoming a range of major obstacles, including vascular access and gas delivery, monitoring and modulating blood and oxygen, pump durability, and achieving membrane biocompatibility to avoid coagulation and infection.

Potential benefit of the ECMO mouse model for synergistic lung studies

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Extracorporeal membrane oxygenation is an effective lifesaving procedure in the treatment of end-stage lung and heart diseases. Despite its proven effectivity in clinic, there are still number of unsolved problems related to complications, limits or challenges of the method. Moreover, experimental ECMO in large animals has its limits due to cost and complexity of whole experimental setup comparable to clinic. Recently, we have developed a novel mouse model of ECMO which was successfully tested using different groups.Due to availability of a number of knock-in/out mice reproducing lung and heart disorders such model might become an indispensable tool for synergistic studies focusing on end-stage conditions.

ECMO circuit consisted of pump, micro-oxygenator, capillary tubing, air-trapper reservoir and cannulation catheters. Total priming volume of the whole circuit was 0,5 mL. Multiple trials and testing allowed achievement of the stable functioning of the murine ECMO.Six C57Bl/6 mice from each group were subjected to 2h, 4h and 6 hours of veno-venous ECMO as an acute model. Another eight animals were successfully "weaned" after 2h of ECMO and sacrificed in 24 h later.Readout parameters included blood gas analysis, blood biochemistry, and histology of lungs, heart, liver, and kidney.

All blood gas parameters were stable during ECMO. The extent of hemodilution was relevant to clinical data obtained from pediatric ECMO. Similar to clinical results signs of multi-organ damage of different extent were seen in lungs, kidney, and liver. Pulmonary damage was limited to coagulopathy, neutrophilic and lymphocytic infiltration. Acute kidney damage was manifested in increased creatinine level and tubular damage in the outer medulla. Increase in liver enzymes with loss of glycogen in liver parenchyma showed signs of a transient hepatic damage. In a chronic model of ECMO, all animals underwent 2 h of ECMO well and were active within 24 hours after the procedure. Histological findings correlated to 2h ECMO in acute setup.

Our model allowed observation of early histological changes in murine organs which were not yet reported for a clinic. Successful "weaning" was achieved due to stable functioning of the ECMO and optimal priming volume. A plethora of genetically modified mice suffering from terminal lung conditions could facilitate to conduct diverse studies on pulmonary research. Moreover, such a model can be used as a reliable in vivo "bridging" protocol for studies focusing on the development of implantable technologies.