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Research Paper

Developing a biohybrid lung – sufficient endothelialization of poly-4-methly-1-pentene gas exchange hollow-fiber membranes

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ABSTRACT

Working towards establishing a biohybrid lung with optimized hemocompatibility, this study analyzed the feasibility of establishing flow-resistant endothelium on heparin/albumin coated poly-4-methly-1-pentene hollow fiber gas exchange membranes (PMP-HFs). The seeding efficiency and proliferation of human cord blood derived endothelial cells (HCBEC) on PMP-HFs were analyzed under static conditions by WST-8 cell proliferation assay and fluorescence microscopy. The HCBEC monolayer integrity under different flow conditions was also assessed. Endothelial-specific phenotype verification, expression activation levels and thrombogenic state markers were quantified by real-time RT-PCR for cell-to-PMP-HF contact under static and dynamic conditions. The results demonstrated the feasibility of establishing a viable, confluent, and flow-resistant endothelial monolayer on the blood-contact surface of PMP-HFs, which maintained a physiological response to TNFα-stimulation and flow conditions. The endothelial phenotype, expression levels of adhesion molecules and thrombogenic state markers were unaffected by cell-to-PMP-HFs contact. These results represent a significant step towards establishing a biohybrid lung.

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1. Introduction

Lung disease currently ranks 4th among the most prevalent causes of death worldwide, with an increasing incidence and prevalence (World Health Organization, 2004; Mannino and Buist, 2007). The impact of end-stage lung diseases on the quality of life of the patients is severe and the levels of associated co-morbidities and mortality are high. This is not least due to the fact that treatment options are very limited and focus solely on addressing symptoms. As no durable long-term assist device for end-stage lung diseases currently exists, lung transplantation remains the only curative therapy option open to patients. However, this is limited to a small group of carefully selected patients (Abouna, 2008; Hosenpud et al., 1998; Bhorade et al., 2000) and also shows a sobering median survival rate of 5.6 years post transplantation (Christie et al., 2010). Generally, in the event of severe respiratory failure, artificial ventilation is required. This provides life-saving gas exchange, but also can cause ventilation-associated lung injury (VALI) (Pinhu et al., 2003), resulting in secondary organ failure (Imai et al., 2003). If artificial ventilation proves insufficient, extracorporeal membrane oxygenation (ECMO) can be used to support gas exchange, but at the cost of rendering patients bedridden and mostly sedated. Both ECMO and mechanical ventilation are known to be significant risk factors for post lung transplantation mortality and numerous major centers worldwide do not consider patients receiving such support as candidates for lung transplantation (Bhorade et al., 2000). Despite well-adjusted anticoagulation therapy during ECMO support, the inevitable contact between the artificial surfaces and the circulating blood leads to platelet and cytokine activation (Kirsch et al., 2005; Li et al., 2005). Within 4 weeks, this results in increased thrombus formation, which eventually leads to a dramatic decrease in gas exchange and, consequently, device failure. Driven by this unmet need, various research strategies are being pursued to improve this ECMO treatment through optimization of the geometry and the fiber arrangement, as well as different coating strategies of the PMP-HF (Nolan et al., 2011) in order to engineer an artificial lung. Artificial lungs are touted as a potential “destination therapy” for awake patients suffering from severe lung failure, who would otherwise require mechanical ventilation or lung transplantation. Nevertheless, common to all these approaches is the fact, that due to the inevitable contact between artificial, and therefore not biocompatible, surfaces and the human cells, foreign body reaction, thrombus formation and inflammation will be initiated sooner or later resulting in the device failure (Nolan et al., 2011). However, based on recent advances made in regenerative medicine, endothelial cell seeding is considered to be an important tool to optimize hemocompatibility. A functional endothelial monolayer could potentially inhibit the adhesion of circulating cells and plasma proteins (McGuigan and Sefton, 2007), maintain hemostasis and regulate blood clotting (Consigny, 2000), thus improving the biocompatibility of artificial surfaces. Poly-4-methyl-1-pentene gas exchange hollow-fibers (PMP-HFs) with covalently bonded heparin/albumin dip-coating on the fiber surfaces are used in the majority of ECMO systems due to their low density, which provide gas permeability levels of tenfold that of polyethylene and polypropylene. Therefore, PMP-HFs provide an important basis for developing biologically active surfaces, which could be incorporated in a biohybrid lung. Hence, the study focused on analyzing the feasibility of endothelializing PMP-HFs. In particular, the seeding properties, cell proliferation and functionality of human cord blood-derived endothelial cells (HCBECs) applied to PMP-HFs were assessed. The influence of different flow conditions on the preservation of a confluent endothelial monolayer onto PMP-HFs was also investigated in a bespoke flow chamber, of which the flow dynamics were analyzed under computational simulations.

2. Materials and methods

2.1. *Endothelialization of PMP-HFs – seeding efficiency and cell proliferation*

HCBECs were isolated and characterized as described previously (Hess et al., 2010). Heparin/albumin coated PMP-HFs were endothelialized as follows: 7×10^6 HCBECs were cultivated with three pieces of PMP-HFs (measuring 2.5 cm × 2.5 cm) in a falcon tube using endothelial growth medium (EBM-2, Lonza) and rotated at 1 rpm and at 37 °C for 96 h, including a daily medium exchange. Subsequently, the PMP-HFs were transferred to EBM-2 filled petri-dishes for a further 48 h of static culture conditions at 37 °C. The cell medium was changed every 24 h. In order to allow a comparison to standard culture conditions, HCBECs were cultivated on tissue culture plates (TCP) in parallel. Cell viability and seeding efficiency were investigated by staining the cells with Calcein acetomethylester (1 μg/mL, Calcein AM, Life Technologies, Invitrogen) combined with trihydrochloride trihydrate (0.5 μg/mL, Hoechst 33342, Life Technologies, Invitrogen) and subsequent analysis by fluorescence microscopy (Axio observer A1 microscope, Zeiss, Germany). As negative control native heparin/albumin coated PMP-HFs were stained using 5-(and-6)-Carboxytetramethylrhodamine succinimidyl ester (100 μM, TAMRA, Life Technologies, Invitrogen) for subsequent fluorescence microscopic analysis. Cell proliferation was measured every 24 h up to 96 h on TCPs and PMP-HFs using the WST-8 Quick Cell Proliferation Assay

Kit II (BioVision), according to the manufacturer's instructions.

2.2. *Analysis of mRNA expression levels of HCBECs seeded on TCPs compared with PMP-HFs*

HCBECs were cultivated on TCPs and PMP-HFs until a confluent endothelial monolayer was formed. In order to subsequently investigate the activation status of the cells, one half of the endothelialized TCPs and PMP-HFs was stimulated with tumor necrosis factor alpha (TNFα) for six hours (10 ng/ml, Bachem), while the other half was left untreated. For RNA-isolation, EBM-2 was removed and the samples were washed three times with D-PBS (Life technologies, United Kingdom). Thereafter, they were trypsinized and the supernatant was removed and stored at −80 °C. According to the manufacturer’s protocol, the RNeasy Mini Kit (Qiagen), including the treatment with RNase-free DNase set (Qiagen), was used to extract the total mRNA. Final RNA concentration