European Journal of Pharmacology 790 (2016) 28–35

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Technologies in kidney development or replacement

Upscaling of a living membrane for bioartificial kidney device

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ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form
24 June 2016
Accepted 6 July 2016
Available online 6 July 2016

Keywords:
Bioartificial kidney device
Living membrane
Functionalized hollow fiber membranes
Conditionally immortalized human renal proximal tubule epithelial cells
Monolayer of functional cells

ABSTRACT

The limited removal of metabolic waste products in dialyzed kidney patients leads to high morbidity and mortality. One powerful solution for a more complete removal of those metabolites might be offered by a bioartificial kidney device (BAK), which contains a hybrid "living membrane" with functional proximal tubule epithelial cells (PTEC). These cells are supported by an artificial functionalized hollow fiber membrane (HFM) and are able to actively remove the waste products. In our earlier studies, conditionally immortalized human PTEC (ciPTEC) showed to express functional organic cationic transporter 2 (OCT2) when seeded on small size flat or hollow fiber polyethersulfone (PES) membranes. Here, an upscaled "living membrane" is presented. We developed and assessed the functionality of modules containing three commercially available MicroPES HFM supporting ciPTEC. The HFM were optimally coated with L-Dopa and collagen IV to support a uniform and tight monolayer formation of matured ciPTEC under static culturing conditions. Both abundant expression of zonula occludens-1 (ZO-1) protein and limited diffusion of FITC-inulin confirm a clear barrier function of the monolayer. Furthermore, the uptake of 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP^+), a fluorescent OCT2 substrate, was studied in absence and presence of known OCT inhibitors, such as cimetidine and a cationic uremic solutes mixture. The ASP^+ uptake by the living upscaled membrane was decreased by 60% in the presence of either inhibitor, proving the active function of OCT2. In conclusion, this study presents a successful upscaling of a living membrane with active organic cation transport as a support for BAK device.

1. Introduction

Within the growing worldwide population of kidney patients undergoing dialysis treatment, mortality (15–20% per year) and morbidity remain high (Vanholder et al., 2003a). One of the reasons could be the limited removal of protein-bound retention solutes (Krieter et al., 2009; Meyer et al., 2005). Their accumulation is strongly associated with the fatal outcome in the patients (Vanholder et al., 2014). In the functional kidney, proximal tubule epithelial cells (PTEC), equipped with a broad range of transporters, mediate the excretion of those solutes. One of the PTEC transporters involved in the excretion of cationic uremic metabolites and drugs is the basolateral organic cation transporter – 2 (OCT2; *SLC22A2*) (Schophuizen et al., 2013).

The development of a PTEC-based bioartificial kidney (BAK) device could improve existing dialysis therapies for the removal of protein-bound uremic retention solutes (Jansen et al., 2014a; Vanholder et al., 2003a). A key requirement for a BAK is the formation of a "living membrane" consisting of a tight monolayer of renal cells with preserved functional organic ion transporters, grown on an artificial porous hollow fiber membrane (HFM). One side of these HFM need to be highly haemocompatible since it would be in contact with blood, whereas the other side should be bioactive to support the formation of a cell monolayer.

Several groups have presented their achievements in upscaled

Abbreviations: ASP^+, 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide; BAK, bioartificial kidney device; BSA, bovine serum albumin; ciPTEC, conditionally immortalized human proximal tubule epithelial cells; CWF, clean water flux; ECM, extracellular matrix; HBSS, Hank's balanced salt solution; HFM, hollow fiber membrane; IgG, immunoglobulin G; Inulin-FITC, fluorescein isothiocyanate (FITC)-labelled-inulin; KHH, Krebs-Henseleit buffer supplemented with HEPES (10 mM); L-Dopa, 3,4-dihydroxy-L-phenylalanine; PES, polyethersulfone; PTEC, proximal tubule epithelial cell; SEM, scanning electron microscopy; SC, sieving coefficient; UT mix, uremic toxin mix; ZO-1, zonula occludens-1

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http://dx.doi.org/10.1016/j.ejphar.2016.07.009
0014-2999/

BAK systems in recent years (Humes et al., 2014; Saito et al., 2011; Tasnim et al., 2010). In terms of materials for the HFM, Polyethersulfone (PES), polysulfone (PSF), polyacrylonitrile (PAN) and cellulose acetate membranes have mostly been evaluated (Ni et al., 2011). Those materials were chosen for their good haemocompatibility properties and limited fouling, and therefore had to be functionalized by various extracellular matrix (ECM) components, such as laminin, polylysine, pronectin, gelatin, or collagen IV (Ni et al., 2011; Schophuizen et al., 2015; Zhang et al., 2009). As for the renal cell lines, the ones originating from humans are preferred to those from other species (Shitara et al., 2006; Tahara et al., 2005). Several groups showed the presence of various markers, indicating that the cells preserved their phenotype (Oo et al., 2011) without characterizing their function (Humes et al., 2004; Oo et al., 2011, 2013; Sanechika et al., 2011; Zhang et al., 2009). Moreover, the most important drawbacks of primary cells are limited availability, low proliferative capacity and donor-to-donor variation.

To overcome these drawbacks, the recently developed and well-characterized conditionally immortalized human PTEC line (ciPTEC) is a suitable candidate to develop an efficient BAK system (Jansen et al., 2014b; Wilmer et al., 2010). The ciPTEC were transduced with human telomerase (hTERT) that limits replicative senescence by telomere length maintenance. In addition, their proliferation is controlled by the temperature sensitive vector SV40tsA58, allowing proliferation at 33 °C and differentiation in mature PTEC at 37 °C. Two of our recent studies proved the concept of using the PTECs for BAK application. In fact, when the cells were cultured on bioactive Polyethersulfone (PES)-based flat sheet membranes (Schophuizen et al., 2015) or small size hollow fibers (Jansen et al., 2015), they presented functional OCT2.

In this work, we investigated the upscaling of this concept and developed modules containing three "living membranes". The transport properties of the polymeric membrane and the quality and function of the grown ciPTEC monolayer were systematically investigated, including the expression of zonula occludens-1 (ZO-1) protein and the diffusion of fluorescein isothiocyanate (FITC)-labelled-inulin (inulin-FITC). Moreover, we also studied the uptake of a specific fluorescent OCT substrate in the presence or absence of the OCT inhibitors (Schophuizen et al., 2013).

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) unless stated otherwise. MicroPES TF10 hollow fiber capillary membranes (HFM) (wall thickness 100 μm, inner diameter 300 μm, max pore size 0.5 μm) were purchased from 3M – Membrana GmbH (Wuppertal, Germany). CiPTEC were cultured as described previously (Wilmer et al., 2010).

2.2. Module preparation

MicroPES HFM were mounted into mini modules composed of Kartell PP T-shaped connectors (Fisher Scientific, Landsmeer, the Netherlands) and PE rigid semi-transparent tubing diameter 6–8 mm (VWR International B.V, Amsterdam, the Netherlands), see Fig. 1. The modules were potted with polyurethane bi-component resin (Intercol B. V, Ede, the Netherlands), allowed to dry for at least 24 h, and cut open. To ensure further ease of use of the modules, we added Luer Lock fittings and caps (Cole Palmer, Metrohm Applikon BV, Schiedam, the Netherland) to the inlets and outlets, and connected them together using silicon tubing (VWR International B. V, Amsterdam, the Netherlands). Three fibers were

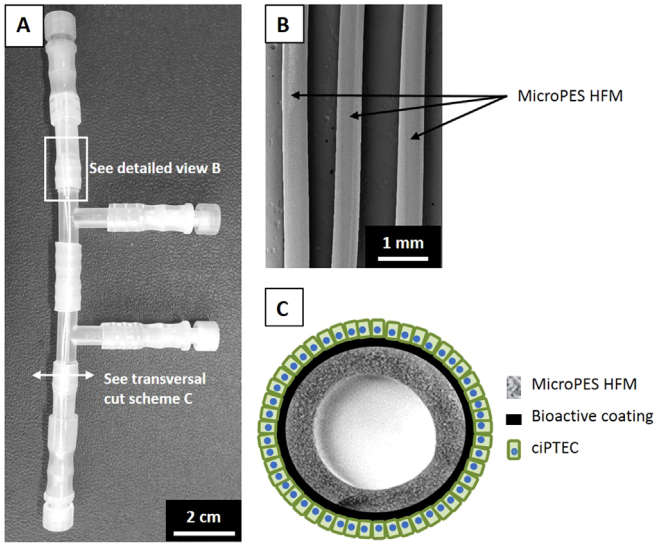

Fig. 1. *Upscaled "living membrane".* (A) Picture of one module used for upscaled "living membrane" model. Three MicroPES hollow fiber membranes (HFM) within a housing composed of PE, PP and silicone parts, Luer Lock fittings and caps. (B) SEM image of three MicroPES HFM. (C) Scheme of a transversal cut of one "living membrane". Not at proportional scale.

mounted in every module, with an effective length of 8.5 ± 0.5 cm and a surface of 4.01 ± 0.25 cm^{-2} available for cell seeding. After cell seeding, extra luminary inlets were supplemented with gas exchange Sartorius Minisart sterile filters (Fisher Scientific, Landsmeer, the Netherlands).

2.3. Membrane sterilization and coating

The bioactive coating was performed on the extraluminal side of the HFM, with slight modifications to the previously established methods (Jansen et al., 2015; Ni et al., 2011). The modules were sterilized using 70% (v/v) EtOH incubation for one h, washed and incubated using sterile 10 mM Tris buffer (pH 8.5) for 1 h. The primary coating component L-Dopa (L-3,4-dihydroxyphenylalanine, 2 mg ml^{-1}) was dissolved in Tris buffer at 37 °C for 45 min. The L-Dopa solution was sterile filtered and injected in the extraluminal space of the module, completely filling it. The primary coating was performed at 37 °C, on a shaking device, for 20 h. Afterwards, the L-Dopa solution was removed; the modules were washed with Hank's balanced salt solution (HBSS) (Fisher Scientific, Landsmeer, the Netherlands) buffer and filled with the second coating component – human collagen IV (25 μg ml^{-1} in HBSS). The coating with collagen IV was performed at 37 °C, on a shaking device, for 2 h. Finally, the collagen IV solution was removed and the modules were washed with HBSS.

2.4. Membrane characterization

The membrane topography of both uncoated and coated HFM was visualized using scanning electron microscopy (SEM), as described previously (Jansen et al., 2015; Schophuizen et al., 2015). We used an OSMO Inspector automated setup (Convergence B. V, Enschede, The Netherlands) to quantify the transport of pure water (Merck Millipore, Billerica, MA) and PBS solutions with 1 mg/ml bovine serum albumin (BSA) and 0.02 mg/ml immunoglobulin G (IgG) solutions through the uncoated and coated cell-free HFMs. The flux through the membranes (J, in l m^{-2} h^{-1}) was plotted as a function of the Transmembrane Pressure (ΔP, in bar). The permeance (L, in l m^{-2} h^{-1} bar^{-1}) was calculated from the slope of this curve. Every pressure step was maintained for