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The performance of primary human renal cells in hollow fiber bioreactors for bioartificial kidneys

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ABSTRACT

Bioartificial kidneys (BAKs) containing human primary renal proximal tubule cells (HPTCs) have been applied in clinical trials. The results were encouraging, but also showed that more research is required. Animal cells or cell lines are not suitable for clinical applications, but have been mainly used in studies on BAK development as large numbers of such cells could be easily obtained. It is difficult to predict HPTC performance based on data obtained with other cell types. To enable more extensive studies on HPTCs, we have developed a bioreactor containing single hollow fiber membranes that requires relatively small amounts of cells. Special hollow fiber membranes with the skin layer on the outer surface and consisting of polyethersulfone/polyvinylpyrrolidone were developed. The results suggested that such hollow fiber membranes were more suitable for the bioreactor unit of BAKs than membranes with an inner skin layer. An HPTC-compatible double coating was applied to the insides of the hollow fiber membranes, which sustained the formation of functional epithelia under bioreactor conditions. Nevertheless, the state of differentiation of the primary human cells remained a critical issue and should be further addressed. The bioreactor system described here will facilitate further studies on the relevant human cell type.

1. Introduction

Bioartificial kidneys (BAKs) consist of a hemofilter in series with a bioreactor unit containing renal proximal tubule cells [1–3]. Both units of BAKs applied in pre-clinical studies and in clinical trials consisted of commercial hemodialysis/hemofiltration cartridges. Cartridges containing polysulfone/polyvinylpyrrolidone (PSF/PVP) hollow fiber membranes were usually applied [2,4–8]. In the bioreactor unit, cells were seeded on the insides of the hollow fibers after coating with an extracellular matrix (ECM). Phase I/II and Phase II clinical trials revealed that BAKs were sufficiently safe and BAK treatment improved long-term survival of critically ill patients with ARF [7,8]. However, the Phase II clinical trial was critically discussed [9], and a subsequent Phase IIb clinical trial was not successful (discussed in [8]). This suggests that further improvements are required.

Human primary renal proximal tubule cells (HPTCs) have been used in clinical trials [7,8], and currently this appears to be the only cell type suitable for clinical applications of BAKs [2,10]. Our results revealed recently that HPTC performance is compromised on PSF/PVP and other synthetic membrane materials [11]. HPTC performance could not be sufficiently enhanced by applying a single ECM coating. However, HPTC performance was substantially improved after applying a double coating consisting of 3,4-dihydroxy-L-phenylalanine (DOPA) and collagen IV [11]. These experiments were performed with flat-bed bioreactors where the performance of cells could be easily assessed.

Due to the limited membrane area, flat-bed bioreactors are not suitable for pre-clinical and clinical studies. Therefore, it would be important to develop experimental systems that allow for the performance of cells seeded on hollow fiber membranes to be easily assessed. Bioreactors with ECM-coated hollow fiber membranes consisting of PSF or other materials have been developed for this purpose, and have been applied in in vitro studies [12–16]. These bioreactors contained cartridges with multiple hollow fibers that required relatively large amounts of cells. The studies were performed with Lewis lung cancer – porcine kidney 1 cells or other animal-derived cells. Such cell types have different substrate requirements than primary human renal cells [10,17], and it is difficult to predict the performance of HPTCs based on results obtained with other cell types.

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In addition to investigating cell performance in hollow fibers, it would be important to develop hollow fibers that are specifically designed for the conditions in the bioreactor unit, which are different from the conditions in the hemofiltration unit. For instance, in the hemofiltration unit, the blood flows in the lumen of the hollow fibers. Accordingly, the skin layer of the synthetic hollow fiber membranes for hemodialysis/hemofiltration is usually on the inside [18,19], whereas the outside often has relatively large pores. In the bioreactor unit, the blood flows on the outsides of the hollow fiber membranes, and thus in this case, it would be preferable to have hollow fiber membranes with the skin layer on the outside. Larger pores on the inner surface of the hollow fibers would facilitate cell attachment and transport processes controlled by the cells.

Herein we developed and characterized hollow fiber membranes specifically designed for the bioreactor unit of BAKs. A bioreactor with single hollow fibers was developed that required only relatively small amounts of cells, and allowed for the thorough characterization of the performance of primary human cells. HPTC performance in the hollow fiber bioreactor was analyzed.

2. Materials and methods

2.1. Fabrication of hollow fiber membranes

Polyethersulfone (PES) (BASF, Ludwigshafen, Germany; average molecular weight (MW) = 51 kDa) and PVP (Merck, Singapore; average MW = 25 kDa) were mixed with N-methyl-2-pyrrolidone (NMP). The final concentrations of PES and PVP were 18 wt% and 10 wt%, respectively, unless otherwise indicated. Fluorinert™ oil FC-3283 (3M, Singapore) was used as bore solution. The flow rates of the dope and bore solutions at the extruder were 0.08 ml/min and 0.05 ml/min, respectively [20]. The coagulation bath consisted of 10 vol% of NMP in water, and wet-spinning was performed at room temperature.

The fibers were immersed in deionized (DI) water for at least 24 h to remove any residual organic solvent, and were flushed with DI water after cutting to the desired length. Hollow fibers used for cell culture were stored in DI water. Samples were freeze-dried for scanning electron microscopy (SEM). The fibers used for solute transport studies and protein adsorption tests were soaked for 24 h in 35 wt% of glycerol, and were assembled into cartridges after drying at 70 °C for 24 h.

2.2. Solute transport and protein adsorption studies

Solute transport studies were performed with cartridges containing 15 hollow fibers with a length of 11 cm. Cartridges were flushed with DI water and then fed through the extra-luminal space with a solution containing 9.14 g/L of bovine serum albumin (BSA), 0.19 g/L of urea and 0.007 g/L of creatinine. The filtrate was sampled, and the solute concentrations were determined by using a Cobas C111 analyzer (Roche, Basel, Switzerland). Pressure sensors (40PC015G, Honeywell, Singapore) were employed to measure the fluid pressures at the inlets and outlets. For protein adsorption studies, a similar set up was used, and hollow fibers were fed either from the extra-luminal space or through the lumen. The feed consisted either of DI water or of 25 g/L of BSA. Protein uptake by hollow fibers was determined by calculating the loss of protein in the feed (i.e. product of the flow rate and the protein concentration difference).

2.3. Pure water permeation flux measurements

Flat PES membranes (Millipore, Bedford, MA, USA) with a molecular weight cut-off (MWCO) of 30 kDa and home-made PES/PVP hollow fibers were mounted either in a flat-bed bioreactor or in a cartridge. DI water was pumped through either the upper chamber of the flat-bed bioreactor or the luminal side of the hollow fibers. When the trans-membrane pressure was stabilized, the permeate volume was measured during a defined period of time. The normalized pure water permeation flux (PWP, L m^{-2} bar^{-1} h^{-1}) was calculated using the following equation:

$$PWP = \frac{Q}{A \bullet \Delta P}$$

where Q is the permeate volume in a predetermined time period (L h^{-1}), A is the effective membrane surface area (m^2) and ΔP is the trans-membrane pressure (bar).

2.4. Cell culture

HPTCs and NIH 3T3 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). HPTCs were cultivated with renal epithelial cell basal medium supplemented with 0.5% fetal bovine serum (FBS) and renal epithelial cell growth kit-BBE (ATCC). HPTCs were used up to passage 5. NIH 3T3 cells were cultivated in Dulbecco's Modified Eagle's Medium with 4500 mg/L of glucose supplemented with 10% FBS. Cell culture media contained 1% of penicillin/streptomycin (Invitrogen, CA, USA). For bioreactor seeding, cells were detached using trypsin-versene and re-suspended in cell culture medium at a concentration of 3×10^6 to 5×10^6 cells/ml.

2.5. Bioreactor

The bioreactor consisted of 3 PES/PVP hollow fiber membranes in a polypropylene housing. Each hollow fiber was attached to gas-permeable tubings (PharMed BPT tubing, Cole–Parmer, Vernon Hills, Illinois, USA) via nylon barbed luer adapters (Cole–Parmer) and hollow fiber holders that were made from 25-g syringe needle adapters (BD, Singapore). A schematic drawing of the bioreactor is shown in Fig. 3. 3-way male lock stopcocks were inserted into the inlet and outlet tubings for collecting samples. A sterile filter (pore size = 0.2 μm) was inserted into the housing for gas exchange. Perfusion was driven by a multi-channel peristaltic pump (Ismatec, Glattbrugg, Switzerland).

2.6. Bioreactor handling and hollow fiber double coating

Bioreactors were perfused (1 ml/min) with 70% ethanol overnight, and subsequently with sterile phosphate buffered saline (PBS) for 8 h. Afterwards, hollow fibers were double-coated with DOPA (Sigma–Aldrich, Singapore) and human collagen IV (Sigma-Aldrich) [11]. DOPA solution (0.2 wt% in 10 mM Tris buffer, pH 8.5) was injected into the hollow fibers, and coating was performed overnight at room temperature. Hollow fibers were rinsed with sterile PBS before injection of 150 μg/ml of human collagen IV solution. Collagen IV coating was performed for 24 h, and hollow fibers were thoroughly rinsed with sterile PBS afterwards. The cells were then injected into the luminal side of the double-coated hollow fibers and were incubated at 37 °C and 5% CO_2 for 90 min. The seeding procedure was performed 4 times with each time rotating the bioreactor by 90° before repeating cell seeding. Afterwards, the extra-luminal space was filled with cell culture medium (~ 100 ml), and the bioreactor was incubated under static conditions for 2 days. From day 3 onwards, the lumen of the hollow fibers was perfused with cell culture medium at a flow rate of 80 μl/min per hollow fiber. Perfusion was continued for 7 days before immunostaining and functional assays were performed. During this period, perfusion was performed with a closed circuit. Each hollow fiber had a cell culture medium reservoir of 100 ml, and the cell culture medium was exchanged after 3 days. During functional assays, perfusion was performed with an open circuit.

2.7. Scanning electron microscopy (SEM)

Both uncoated and double-coated hollow fibers were vacuum-dried overnight and cut to obtain cross-sections or to expose the inner surface. Samples were sputtered with platinum, and SEM was performed with a JSM-7400F field emission scanning electron microscope (JEOL, Tokyo, Japan).

2.8. Immunostaining

Bioreactors were dissembled and hollow fibers were cut into small sections for immunostaining. Samples were washed with PBS and fixed with formaldehyde (3.7% in PBS) for 10 min. After washing with PBS, the cells were permeabilized with 0.5% saponin in PBS at room temperature for 10 min and blocked with 1% BSA (Sigma–Aldrich) for 1 h. The cells were then stained with the following primary antibodies: mouse anti-aquaporin-1 (AQP1) (sc-32737, Santa Cruz, California, USA), mouse anti-URO10 (sc-58889, Santa Cruz), mouse anti-aminopeptidase N (CD13) (ab13448-50, Abcam, Cambridge, UK), mouse anti-glucose transporter-1 (GLUT1) (ab40084, Abcam), rabbit anti-sodium-dependent glucose co-transporter 2 (SGLT2) (ab37296, Abcam), and rabbit anti-proton-coupled peptide transporter 1 (PEPT1) (sc-20653, Santa Cruz). Incubations with the primary antibodies were performed overnight at 4 °C. Samples were washed with PBS and were incubated with fluorescein-conjugated secondary antibodies overnight at 4 °C. Cell nuclei were stained with 4′,6′-diamidino-2′-phenylindole (DAPI). Epifluorescence imaging was performed by using an Olympus BX-DSU microscope (Olympus, Tokyo, Japan).

2.9. Determination of γ-glutamyl transferase (GGT) activity

Cells were perfused for 4 h with an open circuit with phenol red-free medium containing 1 mM of γ-glutamyl-p-nitroanilide (Sigma–Aldrich) and 20 mM of glycylglycine (Sigma–Aldrich) for conditioning. After the conditioning period, the medium was collected at the outlet during the following hour. In addition, samples were taken at the inlet. The absorbance of the collected medium was measured at 405 nm using a microplate reader (Tecan Safire ^{2TM}, Männedorf, Switzerland). The GGT activity of cells was calculated from the standard curve, which was based on known concentrations of p-nitroaniline (Merck, Darmstadt, Germany).