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Extracellular matrix-coated polyethersulfone-TPGS hollow fiber membranes showing improved biocompatibility and uremic toxins removal for bioartificial kidney application

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ABSTRACT

In this study, L-3, 4-dihydroxyphenylalanine and human collagen type IV were coated over the outer surface of the custom-made hollow fiber membranes (HFMs) with the objective of simultaneously improving biocompatibility leading to proliferation of human embryonic kidney cells-293 (HEK-293) and improving separation of uremic toxins, thereby making them suitable for bioartificial kidney application. Physicochemical characterization showed the development of coated HFMs, resulting in low hemolysis ($0.25 \pm 0.10\%$), low SC5b-9 marker level (7.95 ± 1.50 ng/mL), prolonged blood coagulation time, and minimal platelet adhesion, which indicated their improved human blood compatibility. Scanning electron microscopy and confocal laser scanning microscopy showed significantly improved attachment and proliferation of HEK-293 cells on the outer surface of the coated HFMs, which was supported by the results of glucose consumption and MTT cell proliferation assay. The solute rejection profile of these coated HFMs was compared favorably with that of the commercial dialyzer membranes. These coated HFMs showed a remarkable 1.6–3.2 fold improvement in reduction ratio of uremic toxins as compared to standard dialyzer membranes. These results clearly demonstrated that these extracellular matrix-coated HFMs can be a potential biocompatible substrate for the attachment and proliferation of HEK-293 cells and removal of uremic toxins from the simulated blood, which may find future application for bioartificial renal assist device.

1. Introduction

Bioartificial kidney (BAK) is a novel biotechnological approach to better replicate the functions of normal kidneys by incorporating the aspects of living cellular and tissue function to traditional kidneys dialysis systems. The main component of a BAK constitutes hollow fiber membranes (HFMs), which should be compatible with human blood on the inner side and provide a monolayer kidney cells attachment on their outer surface [1,2]. It is also expected for HFMs to show uremic toxins clearance from the impure blood.

Biocompatibility is an important and desired property for a biomaterial, that is, a non-toxic response is expected from a biomaterial with living cells [3]. The compatibility with human blood, and the growth of living kidney cells on the outer surface of HFMs would be useful in developing bioartificial renal assist devices. It is worth mentioning that the separation of uremic toxins including urea, creatinine and phosphorus from the blood is also important for BAK application.

Polyethersulfone (P or PES) is being used to develop HFMs for biomedical applications, particularly kidney dialysis. The favorable properties of PES are good oxidative, thermal, mechanical and hydrolytic stability [4,5]. However, there are literature reports which indicate a need to improve the biocompatibility and uremic toxins clearance of PES HFMs [6–10]. In previous studies, it was experimentally demonstrated that the addition of d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) in HFMs significantly improved the hemocompatibility, cells attachment and proliferation, and separation performance, which are desirable for applications including BAK [10,11]. Furthermore, it is also reported in the literature that coating of extracellular matrix (ECM), e.g., laminin, collagen IV, pronectin-F, entactin/nidogen, perlecan or/and pronectin-L improved the adhesion and proliferation of cells on HFMs [12–15]. In addition to this, the double coating of L-3, 4-dihydroxyphenylalanine (L-DOPA) and human collagen type IV (collagen) significantly improved the attachment and prolifer-

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ation of human renal proximal tubular cells on HFMs [16,17]. It is important to mention that there are only a few studies, to the best of the authors' knowledge, in which biocompatibility of HFMs was evaluated using human embryonic kidney-293 (HEK-293) cells [10,18,19], which is derived from human embryonic kidney cells [20]. The study of the attachment and proliferation of HEK-293 could be useful for the research community working in the field of bioartificial kidney.

The main objectives of the present study were to improve the biocompatibility and uremic toxins clearance of HFMs by coating L-DOPA and collagen on their outer surface. Here, HFMs comprising of TPGS and PES were developed (denoted as TP HFMs). The outer surface of these HFMs was dip-coated with L-DOPA and collagen to obtain coated TP (CTP) HFMs. The physicochemical characterization of the developed HFMs was carried out. The hemocompatibility of HFMs was evaluated, and HEK-293 cells culture study was performed. The transport properties including pure water permeability (PWP), molecular weight cutoff (MWCO) and uremic toxins removal capacity of these HFMs were also measured.

2. Experimental section

In this section, the experimental procedures to develop HFMs, and to coat ECM on the outer surface of HFMs are described. It is followed by presenting the experimental details pertaining to the physicochemical characterization, hemocompatibility evaluation, and HEK-293 cells culture study on the outer surface of the developed HFMs. Finally, the experimental details to measure PWP, MWCO, and uremic toxins clearance are presented.

2.1. Fabrication of HFMs

P and TP HFMs were fabricated as per the details mentioned in a previous study [11]. The process parameters are listed in Table 1. To prepare CTP HFMs, the developed TP HFMs were sterilized by dipping in 70% ethanol overnight, removed from ethanol, and exposed to ultraviolet light for 30 min. HFMs were then thoroughly washed with sterile phosphate-buffered saline (PBS) to remove excess ethanol. Afterwards, TP HFMs with closed ends were dipped in L-DOPA solution (0.2 wt.% in 10 mM Tris buffer, pH ~ 8.5) (Sigma-Aldrich, United States) for 5 min at room temperature. Next, HFMs were rinsed with sterile PBS, and dipped in 150 μg/mL of collagen solution for 5 min. These HFM samples were rinsed with sterile PBS afterwards. These coated TP HFMs are termed as CTP HFMs. Thus, in this study, three HFM samples were considered for the comparison purposes:

(1) P: PES HFMs,
(2) TP: TPGS embedded in PES HFMs, and
(3) CTP: L-DOPA and collagen coated TP HFMs.

Table 1
Process parameters to develop HFMs.

Ambient Temperature (°C)	25		
Dope solution composition	**Sample name**	**PES**	**TPGS**
(wt.%) prepared in NMP	P	20	0
	TP	20	10
Bore solution composition	Deionized (DI) water		
Dope solution temperature (°C)	25		
Bore solution temperature (°C)	25		
Dope flow rate (mL/min)	1.5		
Bore flow rate (mL/min)	1.5		
Air gap (cm)	45		
Coagulation bath composition	DI water		
Rinse bath composition	DI water		
Coagulation bath temperature (°C)	25		
Rinse bath temperature (°C)	25		

Additionally, the commercial dialyzer membranes (F6 HPS HFMs procured from Fresenius Medical Care AG & Co., Germany) were also considered to compare the hemocompatibility and transport properties of the above-developed HFM samples.

2.2. Physicochemical characterization of developed HFMs

Surface morphology of HFMs was studied using field emission scanning electron microscopy (FESEM) (JEOL JSM-7600F, Japan). The energy dispersive X-ray spectrometry (EDS) elemental mapping was performed on the outer surface of CTP HFMs (X-Max 20 mm^2 EDS system, Oxfords Instruments, United Kingdom). The attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was performed on HFMs to record the spectra (3000 Hyperion Microscope with Vertex 80 FTIR System, Bruker, Germany). X-ray diffraction (XRD) patterns of HFMs were recorded in the 2θ range of 6°–50° (scan rate = 3°/min) using a Cu-Kα radiation (k = 1.54178 Å) (Smart Lab, Rigaku, United States). The surface roughness of the outer surface of flattened HFM samples was studied using atomic force microscopy (AFM) (MFP-3D-BIO, Asylum Research, United States). 3 μm × 3 μm area was scanned in the air-tapping mode using SiN probe cantilever (spring constant ~40 N/m). Water contact angle was measured using Digidrop GBX (GBX Instruments, Romans, France). Young's modulus of HFM samples was calculated using Universal Testing Machine (INSTRON®, United States) at an elongation speed of 5 mm/min with load cell of 50 N.

2.3. Hemocompatibility evaluation of HFMs

In BAK devices, HFMs come in contact with human blood, so it is important to evaluate the hemocompatibility (compatibility with human blood) of these HFMs. The hemocompatibility studies include hemolysis, complement activation, blood clotting time, and platelets adhesion and activation. These studies were performed as per the protocols reported for HFMs in the literature [11,19,21,22]. The blood was collected from an informed consent (by extant institutional guidelines) in BD Vacutainer® PLUS plastic plasma blood collection tubes containing 143 USP units sodium heparin (spray-coated) anticoagulant (Becton, Dickinson and Company, United States).

2.3.1. Hemolysis

Erythrocytes were obtained by centrifuging the collected blood at 1250 rpm for 15 min at 10 °C, and discarding the supernatant having platelet-rich plasma (PRP). The erythrocytes were washed 3 times with normal saline solution (NSS). 50% hematocrit was prepared to incubate HFM samples for 1 h at 37 °C and 5% carbon dioxide (CO_2) in an incubator (Thermo Scientific Inc., United States). The centrifugation of the samples and controls (water as positive control and NSS as negative control) was performed for 5 min at 1000x*g*. The absorbance of the samples was taken at 542 nm using UV–vis spectrophotometer (Molecular Devices, United States). The following Eq. (1) was used to calculate hemolysis ratio (HR):

$$HR = \left(\frac{AS - AN}{AP - AN}\right) \times 100 \quad (1)$$

where *AS* stands for the absorbance of sample supernatant, *AN* and *AP* are absorbance of negative and positive controls, respectively.

2.3.2. Complement activation

The centrifugation of the collected blood was performed for 15 min at 1500 RCF to obtain plasma, which was placed in the lumen of HFMs based on equal surface area. The samples were then incubated for 30 min at 37 °C. The recovered plasma was diluted in the ratio of 1:10. MicroVue™ SC5b-9 Plus EIA kit (Quidel Corporation,