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Extracorporeal endotoxin removal by novel L-serine grafted PVDF membrane modules

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ABSTRACT

In this work, two sizes of affinity membrane modules for extracorporeal elimination of endotoxin from blood were prepared by grafting L-serine (Ser) ligand onto PVDF hollow fiber membrane. The chemical modification of membrane surface was verified by X-ray photoelectron spectroscopy (XPS) analysis. The modified fiber's ability to adsorb endotoxins was examined in aqueous solutions with varied pH value and ionic strength, while the protein adsorption of this fiber was investigated in BSA solution. The small-sized module with 159.4 cm^2 effective area was used to remove endotoxin from sepsis patients' plasma in vitro. As results, when the original concentration was 0.42 EU/ml, the endotoxin adsorption capability was 0.058 EU/cm^2 and the removal efficiency was almost 100% for 15 ml, 93.5% and 48.3% for 20 ml and 40 ml samples, respectively. The recovery ratios of total protein and albumin were achieved up to 85.1% and 92.4% correspondingly. The pig model was finally established to evaluate the endotoxin removal efficacy of the scaled up module in direct hemoperfusion. The endotoxin and the inflammation mediator levels decreased after hemoperfusion treatment, indicating the promising potential of this novel adsorber for blood purification of endotoxin.

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1. Introduction

Endotoxin, the lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria, produces serious biological effects on human beings and other mammals when it enters into the blood system, even at extremely low concentration. It is considered as a key factor in the pathogenesis of sepsis or severe sepsis [1]. The rate of severe sepsis hospitalization increases continuously during past decades and the general mortality is extremely high both in the USA and Europe in the intensive care units (ICUs) due to the lack of effective therapies [2,3]. Great efforts have been made on the in vivo treatment, but the clinical outcomes are not satisfying because of poor immunogenicity and inaccessibility of antibodies as well as possible tissue damage induced by drugs and disappointing phase III trial results [4,5]. Hence the direct blood purification method begins to attract attention as a potential promising way.

During the past two decades, hemoperfusion had shown its significance in sepsis therapy as a kind of continuous extracorporeal treatment approach, where the circulating endotoxins and immune mediators in blood could be removed by selective adsorption. Several extracorporeal adsorbers, such as Matisse® adsorber (Fresenius Hemocare Adsorber Technology GmbH, Germany) [6,7], H.E.L.P system (B. Braun, Germany) [8], Toraymyxin (Toray Industries Inc., Japan) [9] and CTR column [10], are now under investigation in clinical applications and have been proved to possess the capability of removing endotoxins from severe sepsis patients' plasma. For instance, over 30,000 sepsis and septic shock patients have been treated by Toraymyxin cartridge in Japan, in which polymyxin B was immobilized onto polystyrene fabric. The cartridge is considered to be able to remove circulating endotoxin and improve hemodynamic dysfunction [9]. Despite the extracorporeal adsorption approach can be an appropriate choice for sepsis therapy, polymyxin B is highly expensive and its nephrotoxicity and neurotoxicity are also crucial issues. Additionally, the main limiting factor is its removal efficiency, as the general reduction of plasma endotoxin level of those reported adsorbers is only 30–40% [7–9], indicating the necessity to develop alternative products which are characterized with low cost, high safety and high efficiency.

To remove effectively the endotoxin from blood, the ligands immobilized on matrices are expected to bind endotoxin

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selectively with a low non-specific adsorption. Endotoxin is chemically composed of a polar polysaccharide chain and a non-polar partially phosphorylated domain called lipid A, which is amphiphilic and is the most conservative part of endotoxin [11]. Most adsorbents are designed to interact with lipid A region through the electrostatic and hydrophobic interactions between the ligands and endotoxin, like diethylaminoethane (DEAE) [12], polymyxin B (PMB) [13], histidine [14], lysine [15], poly(ethyleneimine) (PEI) [16], poly(L-lysine) (PLL) [17], dimethylamine [18] and chitosan [19]. However, the electrostatic and hydrophobic interactions exist simultaneously between those cationic ligands and acidic proteins, which results in high protein loss. Therefore, the ligands which can adsorb endotoxin by other kinds of interactions are desirable. Recently, Wei et al. [20] prepared a series of adsorbents with different amino acid ligands for endotoxin removal, and found that the removal efficiency of L-serine (Ser) was up to 78% in rabbit's serum. The results from the computer simulation clearly showed that a firm cage structure was formed between endotoxin phosphoric residue and Ser adsorbent via three couples of hydrogen bonds with the assist of hydroxyl group on the lateral chain of Ser ligand, NH group on the linkage site between Ser and space arm, and hydroxyl group on space arm, indicating the importance of hydrogen bond and steric effect in the process of Ser ligand binding to endotoxin. Besides, Ser is much cheaper and safer than PMB. Thus, Ser might be suitable for application in the blood system. Nevertheless, the conventional agarose column used in Wei's work limits its practical application due to low sample volume. Therefore, further research on the adsorption module is still required to improve its configuration.

Alternatively, hollow fiber affinity membrane module, with advantages of high flow rate, large throughput, low axial-pressure drop and easy scale-up [21], can also selectively adsorb the target compound when specific ligands are grafted. It has been applied in the depyrogenation operation of protein solutions and DNA samples [22,23]. Some surface-modified hemodialysis hollow fiber membranes are also reported to have ability to remove LPS from blood [24,25]. Compared with traditional adsorbers which are designed as columns packed with functionalized beads, hollow fiber affinity membrane can reduce the total resistance of the module to obtain a stable blood flow, which is beneficial to the hemoperfusion process. Therefore, hollow fiber affinity membrane modules are potential for the practical hemoperfusion application.

Poly(vinylidene fluoride) (PVDF), with good mechanical property and extraordinary chemical stability, has attracted increasing attention as a promising biomaterial due to its excellent biocompatibility [26]. On the other hand, the application of PVDF membrane as the blood-contact material is limited by its hydrophobic nature due to the simultaneous non-specific adsorption of proteins. Our previous work had provided an effective modification method to increase the hydrophilicity of PVDF [27]. The PVDF hollow fibers with hydroxyethyl cellulose (HEC) coating have shown a low non-specific adsorption in human plasma. Therefore, the PVDF hollow fiber membranes are grafted with L-Ser after hydrophilic modification and further studied for the endotoxin removal from blood.

In this work, the novel adsorbents were prepared by using PVDF hollow fibers as supported material and L-Ser as the ligand. The static specific adsorption behavior of the adsorbent was then studied in an aqueous solution, and the non-specific adsorption was investigated using BSA as the objective protein. Afterwards, the endotoxin clearance efficiency and the hemocompatibility of the affinity membrane module were evaluated using the sepsis patients' plasma where the endotoxin level was around 1 EU/ml. Finally the hemoperfusion experiment was carried out using pig model and the therapy results were further investigated.

2. Materials and methods

2.1. Materials

The PVDF hollow fiber membranes used in this study were prepared in our laboratory. The inside and outside diameters of the fibers were 0.8 and 1.1 mm respectively, with the average pore size of 0.18 μm, the porosity of 85%, and the water flux (0.1 MPa) of 69.71 ml/cm^2 min. HEC was purchased from Fluka (Buchs, Switzerland). 1, 6-Hexanediamine (HDA), as the space arm, was from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). While, amino acid L-serine was bought from Shanghai Kangda Co. (Shanghai, China). Deionized water was used throughout the experiments.

Bovine serum albumin (BSA, Mw 67,000, pI = 4.7) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *Escherichia coli* 0111: B4, L2630 (Sigma–Aldrich, USA) was used as the endotoxin standard samples. Quantitative chromogenic tachypleus amebocyte lysate (TAL) for endotoxin detection (the minimum detection limit is 0.01 EU/ml) and endotoxin-free water were purchased from Xiamen Horseshoe Crab Reagent Manufactory Co., Ltd. (Xiamen, China). Human plasma was supplied by volunteer sepsis patients in Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University.

Male domestic pigs weighing 25–30 kg were used as the animal experimental body. The animal preparation was referred to the previous study [28]. ELISA kits for tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) testing were purchased from Bluegene Biotech CO., Ltd. (Shanghai, China).

All other chemical and biological reagents were of analytical grade from local chemical reagent companies. All the glass apparatuses were heated for 4 h at 400 °C and plastic apparatuses were immersed in 30% H_2O_2 to keep pyrogen-free conditions throughout the endotoxin adsorption experiments.

2.2. Affinity membrane preparation and characterization

PVDF membrane was chemically modified as shown in Fig. 1 [27]. The PVDF hollow fibers were treated with 4% (w/v) $KMnO_4$ and 3 mol/L KOH at 80 °C for 30 min, subsequently were immersed in 5% (v/v) H_2SO_4 and 5% (w/v) $NaHSO_3$ at room temperature till they turned white (step 1). The hydroxylated membranes were then shaken in 1.5% (w/v) HEC (pH 6–7, adjusted by NaOH) at 90 °C for 15 min to increase the reactive hydroxy groups of the surface. After dried, the membranes were washed with 0.5 mol/L $NaHCO_3$ at 90 °C (step 2). Thereafter, the HEC-bound membranes were treated with 4:1 (v/v) mixture of 1.0 mol/l NaOH and chloroepoxy propane at 60 °C for 1 h (step 3). Then the membranes were shaken in 5% (w/v) 1,6-hexanediamine (HDA) at 60 °C for 2 h to fix spacer arms (step 4). After being treated with chloroepoxy propane again (step 5), the resulting membranes were shaken in 3.0 mg/ml L-serine (Ser) at 45 °C for 24 h (step 6), which was prepared in 0.2 mol/L sodium phosphate buffer (pH 7.2). Finally, the membranes were washed with 0.2 mol/L sodium phosphate buffer and deionzied water, followed by drying process and stored for later use.

The surface properties of PVDF membrane and PVDF-Ser affinity membrane were analyzed by X-ray photoelectron spectroscopy (XPS) (AXIS-UlTRA DLD Kratos Co., England). The amount of amino acid coupled on the PVDF-Ser membrane was determined by measuring the initial and final concentrations of the serine solution in step 6 using the ninhydrin method [29]. The amount of Serine was calculated to be 4.42 μmol/cm^2 membrane.

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