Advanced Membranes 3 (2023) 100067

Contents lists available at ScienceDirect

Advanced Membranes

journal homepage: www.keaipublishing.com/en/journals/advanced-membranes

Fabrication of anti-leakage Hyflon AD/Poly(4-methyl-1-pentene) hollow fiber composite membrane for an extra-corporeal membrane oxygenation (ECMO) system

Ting He [a], Yinchao Jin [a], Jie Li [a], Zhaoyun Sun [b], Zhaohui Wang [a], Qingguo Li [b], Rizhi Chen [a], Zhaoliang Cui [a,*], Young Moo Lee [c,**], Enrico Drioli [d]

[a] State Key Laboratory of Materials-Oriented Chemical Engineering, National Engineering Research Center for Special Separation Membrane, College of Chemical Engineering, Nanjing Tech University, Nanjing, 211816, China

[b] Cardiovascular Center, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, 210000, China

[c] Department of Energy Engineering, College of Engineering, Hanyang University, Seoul, 133-791, Republic of Korea

[d] Institute on Membrane Technology, National Research Council of Italy, ITM-CNR, Rende, Italy

ARTICLE INFO

Keywords:
Poly(4-methyl-1-pentene) (PMP)
Anti-leakage
Hemocompatibility
Hyflon AD60

ABSTRACT

Extra-corporeal membrane oxygenation (ECMO) systems can perform the roles of the human heart and lungs to realize extra-corporeal oxygenation of blood. This system mainly depends on the gas-blood exchange membrane, the quality of which impacts the oxygenation performance. Currently, the most widely used gas-blood exchange membrane is made of poly(4-methyl-1-pentene) (PMP) hollow fibers. However, plasma leakage often occurs during clinical applications, which decreases the oxygenation performance and the service life and may endanger the patient's life in serious cases. In this work, Hyflon AD/PMP hollow fiber composite membranes were prepared by coating Hyflon AD on the surfaces of PMP hollow fibers to form ultra-thin, dense layers. Compared the plasma leakage time of the composite membrane with that of the pristine PMP membrane, the Hyflon AD60 layer showed great improvement in anti-leakage performance. The Hyflon AD60/PMP hollow fiber composite membrane possessed lower platelet adhesion and protein adhesion than that of the PMP membrane, indicating better blood compatibility of the Hyflon AD60 membrane. Cytotoxicity experiments were conducted to further confirm the biosafety of Hyflon AD60 as a blood contact medical membrane material. Gas permeance and oxygenation performance of the Hyflon AD60/PMP hollow fiber composite membrane were tested to ensure gas exchange efficiency during the gas separation process. Therefore, the optimized Hyflon AD60/PMP hollow fiber composite membrane has potential for clinical use.

1. Introduction

ECMO systems are high-end medical equipment for treatment of critically ill patients. They play a crucial role in treatment of patients with cardiopulmonary impairment caused by infectious diseases, such as Coronavirus Disease 2019 (COVID-19), Influenza A, severe acute respiratory syndrome (SARS), and others [1–3]. As the core material of the membrane oxygenator, the gas-blood exchange membrane is a barrier separating the blood from the gas phase and is the site of blood oxygenation [4,5]. The surface microstructure of this membrane has a significant influence on gas migration rate and plasma leakage. Commercial gas-blood exchange membrane materials have been developed over three generations using materials in the order of silicone rubber, polypropylene (PP), and poly-4-methyl-1-pentene (PMP) [6–8]. First-generation solid silicone membranes have advantages of good hemocompatibility and low plasma leakage but disadvantages of difficulty venting, large prefilling volume, and large differential pressure across the membrane layer [9]. Second-generation microporous PP hollow fiber membranes solved the problem of difficult venting, although the possibility of plasma leakage increased due to the micropore structure [10,11]. Third-generation PMP hollow fiber membranes combine the advantages of the previous generations, though there are few reports on PMP membrane technology and large-scale preparation [12,13].

The current method of preparing PMP hollow fiber membranes is the

* Corresponding author.
** Corresponding author.
E-mail addresses: zcui@njtech.edu.cn (Z. Cui), ymlee@hanyang.ac.kr (Y.M. Lee).

https://doi.org/10.1016/j.advmem.2023.100067
Received 10 June 2023; Received in revised form 16 August 2023; Accepted 24 August 2023
Available online xxxx

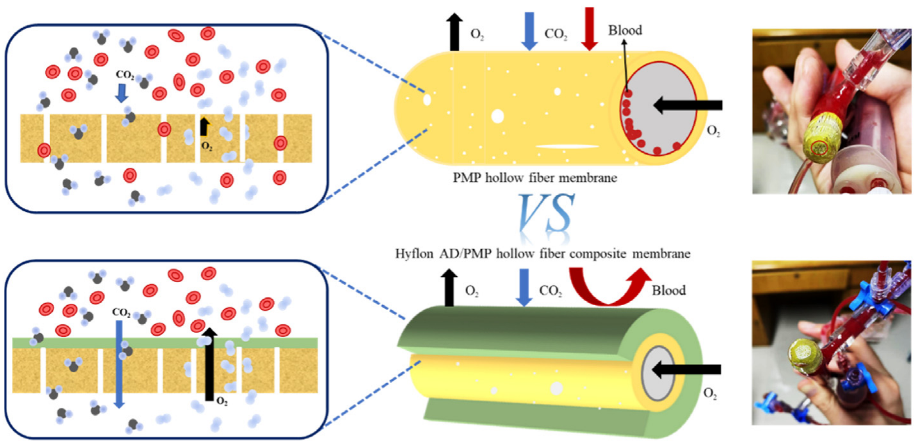

Fig. 1. Preparation schematic of a Hyflon AD/PMP hollow fiber composite membrane.

thermally-induced phase separation (TIPS) process [14], which forms fine defects on the PMP outer surface to ensure the open pore structure and gas permeance. However, such fine defects lead to problems when the PMP hollow fiber membranes are directly applied for clinical use. The two most serious problems are plasma leakage and poor blood compatibility, which have a significant impact on the long-term performance and service life of gas-blood exchange membranes [15–17]. A dense PMP hollow fiber surface layer needs to be prepared to meet the required oxygenation performance and leak-proof performance of the gas-blood exchange membranes.

Hyflon AD is a perfluorinated amorphous glassy copolymer containing 2,2,4-trifluoromethoxy-1,3-dioxole (TTD) and tetra-fluoro ethene (TFE) [18,19]. The component TFE provides high selectivity for He/CH_4 and He/H_2 [20], while the perfluorodioxane solution disrupts the TFE crystallization to form large fractional free volume (FFV) [21–23]. Jalal et al. prepared hydrophobic Hyflon AD/PVDF membranes for dehydration of butanol by osmotic evaporation [24]. Gugliuzza et al. chose PVDF and Hyflon AD 60X as feedstocks to prepare Hyflon/PVDF composite membranes [25]. Meanwhile, our group previously designed highly hydrophobic Hyflon/PVDF composite hollow fiber membranes by coating Hyflon AD on polyvinylidene fluoride (PVDF) membranes. This allowed membrane application in a distillation process, a crystallization process, and a condenser process [26–29]. Hyflon AD is a dense membrane layer with good performance for gas separation [30–33]. Therefore, coating Hyflon AD onto the surface of a PMP hollow fiber can cover defects on the PMP membrane surface to decrease the probability of liquid passing through the PMP membrane surface or of wetting the PMP membrane [34], further prolonging the service life.

In this work, a PMP hollow fiber was homogeneously coated with Hyflon AD solution on the outer surface to prepare long-lasting and leak-proof Hyflon AD/PMP hollow fiber composite membranes, as shown in Fig. 1. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transformation infra-red (FT-IR) spectroscopy, and contact angle measurements were utilized to study the morphologies and physicochemical properties of PMP membrane and Hyflon AD/PMP hollow fiber composite membranes. Gas performance of PMP membrane and Hyflon AD/PMP hollow fiber composite membranes was studied under different membrane preparation conditions (three kinds of Hyflon AD, coating concentration, and coating time). Anti-leakage time and cytotoxicity experiments were also conducted to evaluate the serve life and biosafety of the pristine PMP membrane and the three Hyflon AD/PMP hollow fiber composite membranes. We also sought to compare the oxygen exchange rate and pressure decrease of the optimized Hyflon AD60/PMP hollow fiber composite membrane and the pristine membrane to determine if they meet the requirements for clinical use.

Table 1
The types and intrinsic viscosities of Hyflon AD powders [23].

Type	Intrinsic Viscosity Unit/cc/g
Hyflon AD40H	120 ± 30
Hyflon AD40L	40 ± 10
Hyflon AD60	40 ± 10

2. Experimental methods

2.1. Materials

Poly (4-methyl-1-pentene) (PMP) hollow fiber membranes were provided by Jiangsu Aikemo High-Technology Co., Ltd (China). Tributyl O-acetylcitrate (ATBC) (Purity: AR) and bovine serum albumin (BSA) were purchased from Aladdin (Nanjing, China). Anhydrous ethanol and glycerin were obtained from Sinopharm (Nanjing, China). All chemicals were used as received and were not further purified. Hyflon AD (Hyflon AD40H, Hyflon AD40L, Hyflon AD60) materials were supplied by Solvay Specialty Polymer (Bollate, Italy). Phosphate buffer saline (PBS, pH = 7.4) and Rhodamine 123 were purchased from Solarbio (Beijing, China). The BCA Protein Assay Kit was provided by Cwbio (Nanjing, China). Swine blood (EDTA anticoagulation) was supplied by Yuanye (Shanghai, China). Pure water was used in this work. Our collaborators from Nanjing Medical University provided artificial blood, fresh human whole blood, MET-5A cells, and experimental animals (rabbits) according to ethical requirements.

2.2. Fabrication of Hyflon AD/PMP hollow fiber composite membranes

There are three kinds of Hyflon AD copolymers depending on the contents of 2,2,4-trifluoro-5-trifluorometoxy-1,3-dioxole (TFE) and tetrafluoroethylene (TTD) [22]. The properties of the three Hyflon AD materials (Hyflon AD40L, Hyflon AD40H, and Hyflon AD60) are listed in Table 1 [23]. Hyflon AD40 is available in both a high-molecular weight (Hyflon AD40H) and a low-molecular weight (Hyflon AD40L) version. The Hyflon AD/PMP hollow fiber composite membranes were prepared by coating a Hyflon AD layer onto the surface of PMP hollow fibers. The specific preparation processes of Hyflon AD/PMP hollow fiber composite membranes were as follows. A polymeric coating solution was formed by mixing 0.5 wt% Hyflon AD in Novec HFE-7100 Engineered Fluid at 60 °C. The PMP hollow fiber membranes were directly placed into the Hyflon AD coating solution for 10 min and then dried in an oven at 40 °C for 12 h. Finally, the three kinds of Hyflon AD/PMP hollow fiber composite membranes were obtained, and their performances were studied.

Table 2
Surface free energy of test liquids at 20 °C.

Test liquid	γ_L^p	γ_L^d	γ_L	γ_L^p/γ_L^d
Water	51.00	21.80	72.80	2.36
Diiodomethane	2.30	48.50	50.80	0.05

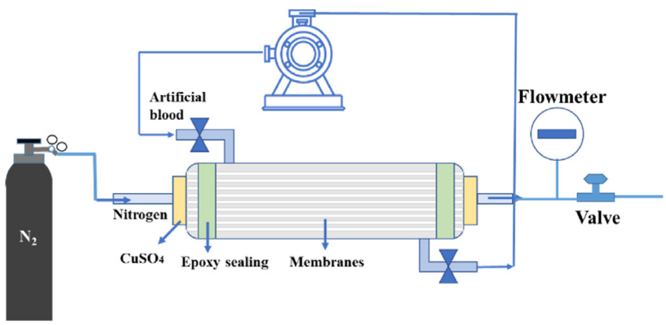

Fig. 2. Schematic diagram of the anti-leakage time experiment device.

Various ordered structures of polymers chains form through evaporation of solvent when applying a Hyflon AD coating layer [35]. Therefore, the different assembly structures with different ratios of TFE and TTD in Hyflon AD will vary and affect the gas permeance and surface properties of the Hyflon AD/PMP hollow fiber composite membranes [24].

2.3. Characterization

The surface and cross-sectional morphologies of PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes were characterized by SEM (Hitachi S-4800, Japan). A contact angle meter (DropMeterA-100P) was applied to test the surface contact angle of the homemade membranes and to calculate surface energy. FT-IR, (NICOLET 8700, Japan) and atomic force microscopy (AFM, Miniflex 600, Japan) were selected to characterize the chemical properties and the surface roughness of PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes, respectively.

2.4. Gas permeation test

Gas separation performance of PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes was tested using a homemade gas permeation device. At a certain temperature, pure gas (1 bar) was passed through the membrane module, and the permeance of different gases was measured by a soap bubble flowmeter. The formula for gas permeation is as follows:

$$\left(\frac{P}{l}\right)=\frac{Q}{A\Delta P} \tag{1}$$

where (P/l) is the gas permeance in mL/(cm^2•min•bar), P is the gas permeability in mL/(cm^3•min•bar), l is the separation layer thickness in cm, Q is the volume flow rate of the gas permeating through the membrane at standard temperature and pressure in cm^3/s, A is the effective area of the permeable membrane in cm^2, and ΔP is the pressure difference on the two sides of the membrane in cm•Hg. Each type of sample was measured five times, and the average value and standard deviation were calculated for analysis.

2.5. Surface energy

Following the Owens-Wendt equations (Eq. 2 and Eq. (3)), the surface free energy of the liquid and its γ_L^d, γ_L^p component values and polarity (using water and diiodomethane as solvents) are shown in Table 2.

$$\gamma_L(1+\cos\theta)=2\left(\gamma_S^d\gamma_L^d\right)^{1/2}+2\left(\gamma_S^p\gamma_L^p\right)^{1/2} \tag{2}$$

$$\gamma_s=\gamma_S^d+\gamma_S^p \tag{3}$$

where γ_S and γ_L are the surface free energy of the solid and the liquid, respectively. In addition, γ_S^d and γ_S^p are dispersion force and polar force terms of the solid, respectively; while γ_L^d and γ_L^p are dispersion force and polar force terms of the liquid.

2.6. Anti-leakage time measurement

The anti-leakage test process is shown in Fig. 2. The shell-sides of the PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membrane modules were filled with artificial blood. Nitrogen (N_2) was purged into the lumen side of the PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes. The inlet and outlet of the PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membrane modules were equipped with drying tubes containing anhydrous copper sulfate powder. N_2 first was passed through anhydrous copper sulfate powder to remove any residual water vapor. The discoloration time of anhydrous copper sulfate was observed in the drying tube at the outlet of the membrane module and was defined as the anti-leakage time. Three parallel tests on the same batch of membranes were conducted, and the average value was calculated for analysis.

2.7. Biocompatibility evaluation

2.7.1. Protein adsorption measurements

Neutral BSA protein was applied for the protein adsorption test of PMP hollow fiber membranes and Hyflon AD/PMP hollow fiber composite membranes. The protein adsorption test was performed using a BCA protein kit staining, and the absorbance values of sample solutions were measured using a microplate reader (Thermo, USA) using the following steps.

(1) Preparation of 0.5 g/L protein standard solution. Five standard solutions at 2 mL were prepared with concentrations ranging from 0.1 to 0.5 g/L. These solutions were used to determine the standard curve of protein adsorption.
(2) Protein adsorption experiment. The same 2 mL amount of 0.5 g/L protein solution was transferred into a 24-well plate. PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes with an area of 0.5 cm^2 were immersed in the protein solution and incubated at 37 °C for 1 h.
(3) Preparation of BCA working solution. Protein adsorption was analyzed via the BCA method. BCA working solution was prepared according to the volume ratio of BCA reagent A and BCA reagent B (50:1).
(4) Determination of protein adsorption. For this, 20 μL of each protein solution from the 24-well plate was moved to a 96-well plate, and 200 μL of each BCA working solution was added. The 96-well plate was incubated in a shaker at 37 °C for 30 min, and the absorbance of the sample solution at 562 nm was measured using a microplate reader. Finally, the protein concentration in the solution was obtained based on the standard curve. Three parallel experiments were performed for each sample.

2.7.2. Platelet adhesion experiment

The platelet adhesion test is an important means of determining the blood compatibility of membrane materials. When blood and membrane material come into contact, plasma proteins are adsorbed on the membrane surface, red blood cells rupture (hemolysis) and are adhered to by platelets, which aggregate and deform to activate coagulation and

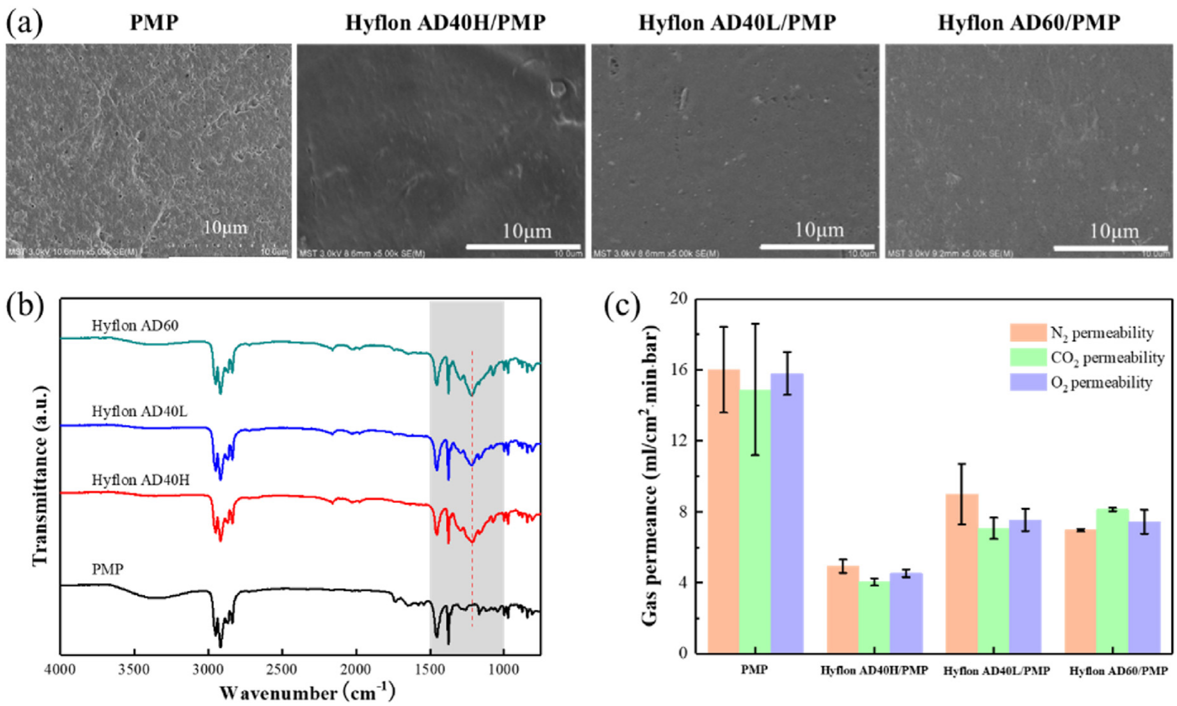

Fig. 3. (a) SEM images of top surface, (b) FT-IR spectra and (c) gas permeance of the PMP hollow fiber membrane and three kinds of Hyflon AD (40H, 40L, 60)/PMP hollow fiber composite membranes.

fibrinolytic systems and eventually form a thrombus. The steps of the platelet adhesion test are as follows.

Platelet-rich plasma (PRP) was prepared by centrifugation (1500 rpm, 15 min) of fresh human whole blood. Next, 100 μL of PRP was added to the wells of a 24-well plate, into which PMP hollow fiber membrane and Hyflon AD/PMP hollow fiber composite membranes with areas of 0.5 cm^2 were immersed and incubated at 37 °C for 1 h in humid air. After washing three times with PBS, the samples were fixed with 2.5% glutaraldehyde in PBS for 1 h.

(1) To facilitate SEM characterization, the samples were dehydrated in 50%, 75%, 90%, and 100% ethanol/PBS mixed solution for 15 min each. After critical point drying, the samples were sputtered with gold for field emission scanning electron microscopy (FE-SEM) characterization. Three parallel experiments were conducted.
(2) The actin backbone of adherent platelets was washed three times with PBS and stained with rhodamine 123 for fluorescence microscopy observations. Staining was performed in a dark room for 15 min and was followed by three washes with PBS. Fifteen randomly chosen platelet surface positions were analyzed with fluorescence microscopy for statistical analysis. Fluorescence microscopy images of all samples were obtained over the same exposure time.

2.7.3. Hemolysis test

Hemolysis is the phenomenon of rupture and lysis of red blood cells when they encounter exogenous substances. We assessed the degree of destruction of red blood cells by chemicals such as bile acid salts in the exogenous substances of membrane filaments. The hemolysis rate in this work reflects the presence of chemicals in the membrane filament that destroy blood cells upon contact. If the hemolysis rate is too high, the membrane filaments will trigger changes in the physicochemical properties of blood cells during use, leading to hemolysis and other phenomena. This test was conducted in accordance with ISO 10993 and GB/T16886 in China.

2.7.4. Cytotoxicity test

In vitro cytotoxicity tests simulate the growth environment of living organisms in an isolated state to detect cell lysis, cell growth inhibition, and other toxic effects of medical devices and biological materials after contact with body tissues. In vitro cytotoxicity tests are some of the most important indicators in biological evaluation of medical devices and are mandatory for clinical application. In this study, we selected the CCK-8 method to detect cell activity as a highly sensitive, non-radioactive colorimetric assay for measuring the number of living cells in cell proliferation or toxicity assays. After PMP hollow fiber and Hyflon AD/PMP hollow fiber composite membranes were placed in full contact with MET-5A cells for 24 h and cultured for 30 h, the light absorption value at 450 nm was measured with a microplate reader. The cell survival rate of the blank group was calculated as a control.

2.8. In vitro oxygenation test

In medical treatment, systems of hollow fiber membranes can perform the function of human lungs. In vitro oxygenation is key for testing the exchange of carbon dioxide and oxygen in blood through the membrane separation process. In this study, the oxygenator assembly was prepared with poly(vinyl chloride) (PVC) tubing as the shell material to compare the oxygenation performance of pristine PMP hollow fiber membranes and that of Hyflon AD60/PMP hollow fiber composite membranes. The effective area of polymeric membranes was 0.3 m^2 in the oxygenator assembly. When the membrane filaments were circulated internally with oxygen and externally with simulated fluid (artificial blood), the properties of the membrane were measured during blood separation, including oxygen exchange rate and pressure decrease. PMP hollow fiber membranes and Hyflon AD/PMP hollow fiber composite membranes were added to PVC pipes according to the selected number and length of membrane filaments. The above membranes were divided into pristine and modified groups to study the effect of membrane type on oxygen exchange performance and pressure decrease after glue sealing, glue cutting, and leak detection.

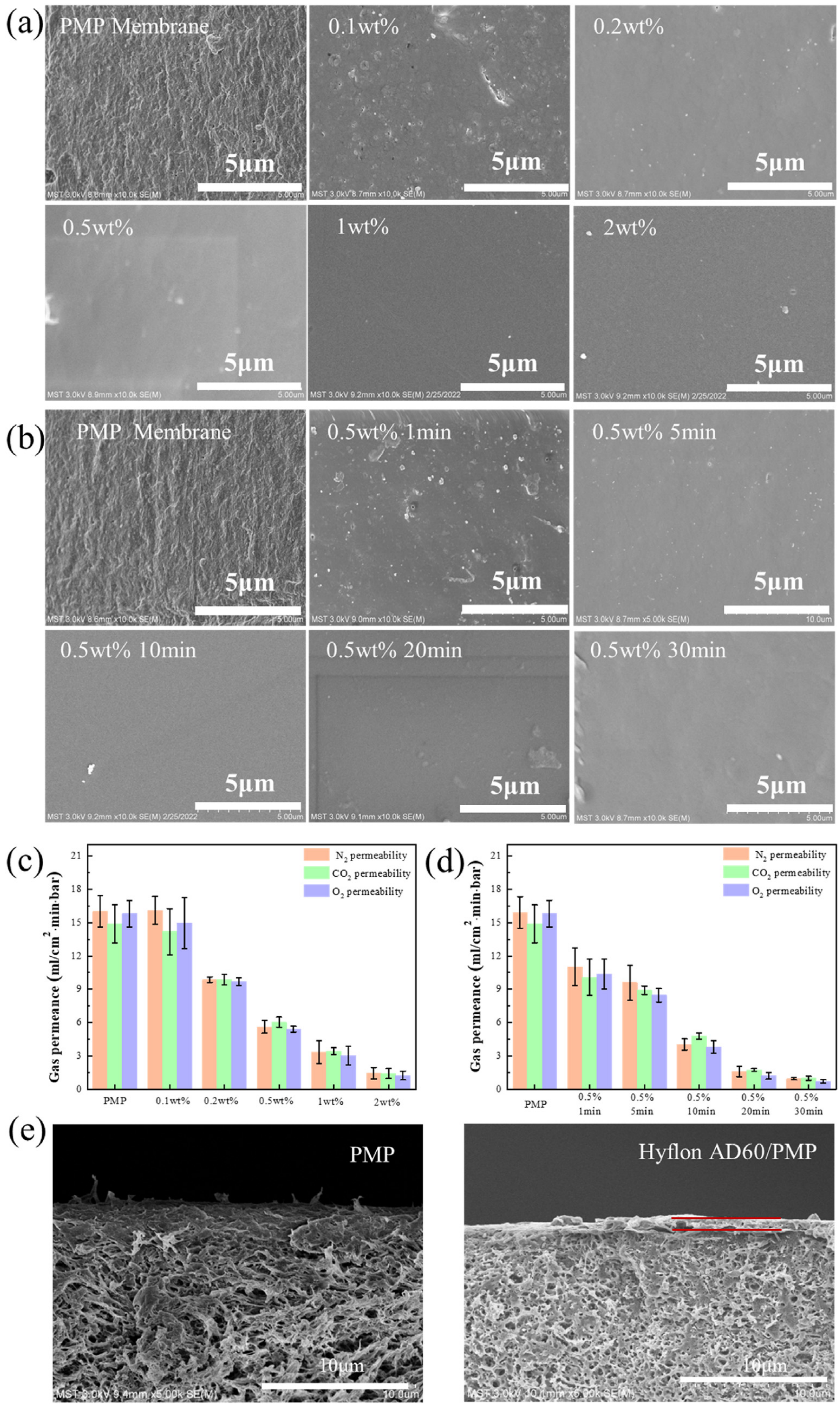

Fig. 4. (a and b) SEM images of top surface and (c and d) gas permeance of PMP hollow fiber membrane and Hyflon AD60/PMP hollow fiber composite membranes with different coating concentrations and times. (e) SEM images of cross-section of the PMP hollow fiber membrane and the optimized Hyflon AD60/PMP hollow fiber composite membrane.

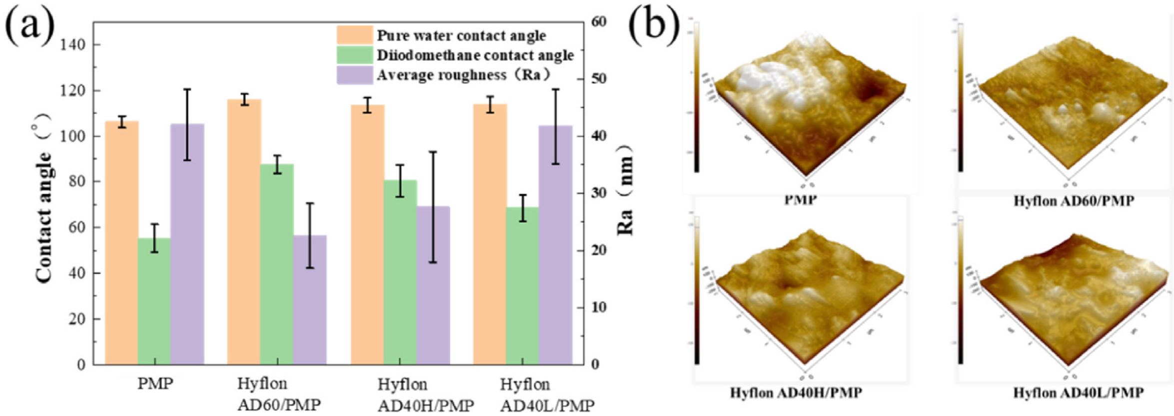

Fig. 5. (a) Contact angle, and (b) surface roughness of the PMP hollow fiber membrane and Hyflon AD/PMP composite hollow fiber membranes.

3. Results and discussion

3.1. Characterization of the membranes

SEM images of top surface of PMP hollow fiber and three Hyflon AD/PMP hollow fiber composite membranes were showed in Fig. 3a. It can be clearly seen that the Hyflon AD coating layer significantly reduces surface defects on the PMP hollow fiber substrate. Compared with the FT-IR spectra of a PMP hollow fiber membrane in Fig. 3b, the new coated membrane showed a new C–F absorption peak at 1214 cm^{-1}, verifying successful coating of the polymeric Hyflon AD layer. As in Fig. 3c, the Hyflon AD60/PMP hollow fiber composite membrane exhibited higher CO_2/O_2 selectivity than the PMP hollow fiber membrane and other Hyflon AD40/PMP hollow fiber composite membranes. It is well known that the transmission rate of CO_2 in human lungs is twice that of O_2 [36–38]. The high selectivity of membrane materials to CO_2 in the in vitro oxygenator will improve oxygenation performance in practical applications. Therefore, polymeric Hyflon AD60 is suitable for preparing composite membranes for ECMO applications.

The effect of Hyflon AD60 concentrations from 0.1 wt% to 2 wt% was investigated on membrane performance. As shown in Fig. 4a-b, when the concentration of Hyflon AD60 coating solution was less than 0.5 wt%, defects remain on the pristine PMP membrane surface. After this concentration, the membrane surface appeared smooth and free of defects. With an increase in coating time, the membrane surface gradually became smooth. As shown in Fig. 4c, the oxygen permeance of the PMP hollow fiber membrane was 16 mL/cm^2·min·bar. As the coating concentration increased, the gas permeance exhibited a decreasing trend. At a coating concentration of 0.5 wt%, the oxygen permeance was half that of the pristine PMP hollow fiber membrane, 5 mL/cm^2·min·bar. It is important to emphasize that we tend to consider membrane materials with high gas permeance when they are applied in traditional chemical applications. However, the situation is different when the membrane is utilized in oxygenator systems. Excessive gas permeance can increase gas content in the blood, which cannot be completely absorbed by the body, resulting in a large amount of foam. The body cannot exchange gas properly in the presence of such foam, and a gas embolism is formed. Therefore, in clinical use, gas permeance is generally required to be less than 10 mL/cm^2·min·bar. Referring to the physical needs of patients during clinical treatment, we chose a 0.5 wt% coating concentration and 10 min of coating time for the subsequent experiment. Therefore, we observed the cross-section of the PMP hollow fiber membrane and the Hyflon AD60/PMP hollow fiber composite membrane by SEM characterization. As can be seen in Fig. 4e, the cross-section of PMP hollow fiber was displayed a porous microstructure. After optimized membrane fabrication conditions, the thickness of the Hyflon AD60 separation layer on the outer surface of PMP hollow fiber was about 1 μm.

Table 3
Surface energy of the PMP hollow fiber membrane and Hyflon AD/PMP composite hollow fiber membranes.

Type	PMP	Hyflon AD60/PMP	Hyflon AD40H/PMP	Hyflon AD40L/PMP
Surface energy-γ_s(mN/m)	32.96	15.05	17.56	25.44

3.2. Surface properties of the membranes

As shown in Fig. 5a, the contact angle and surface roughness of the PMP hollow fiber membrane and the Hyflon AD/PMP hollow fiber composite membranes were tested. Contact angles of all Hyflon AD membranes (AD40H, AD40L, and AD60) increased in comparison to that of the pristine PMP membrane, implying higher hydrophobicity of Hyflon AD/PMP hollow fiber composite membranes. This enhanced hydrophobicity makes it more difficult for human blood to wet the surface and pores of a PMP hollow fiber membrane, decreasing the possibility of blood leakage, consistent with the literature [39]. Meanwhile, the surface roughness of the Hyflon AD/PMP hollow fiber composite membranes was lower than that of the pristine PMP membrane in Fig. 5b, which helps delay protein adhesion and thrombus generation during the gas-blood exchange process. We calculated the change of surface energy among PMP membranes and Hyflon AD/PMP hollow fiber composite membranes via the contact angle and the Owens-Wendt equations (Eq. 2 and Eq. (3)). As can be seen in Table 3, compared with the pristine PMP membrane, the surface energy of three kinds of Hyflon AD/PMP hollow fiber composite membranes is significantly reduced, indicating that the lower surface energy of the Hyflon AD membrane surface can reduce protein contamination [40].

3.3. Anti-eakage time of the membranes

As showen in Fig. 6a, the anti-leakage time experiment was carried out with the PMP hollow fiber membrane and three kinds of Hyflon AD/PMP hollow fiber composite membranes. The contact angle test confirmed that the hydrophobicity of the Hyflon AD material weakened the water adhesion on the surface of the membrane, increasing the time to leakage. As shown in Fig. 6b, the average anti-leakage times of all Hyflon AD membranes (AD40H, AD40L, and AD60) were longer than that of the pristine PMP membrane (10 days), implying that the Hyflon AD/PMP composite membranes have good interfacial interaction between Hyflon AD layer and PMP substrate. In particular, the average anti-leakage time of the Hyflon AD60 membranes was 22 days, which is 2.2 times that of the pristine PMP membrane and exceeds the 14 days reported of commercial membrane.

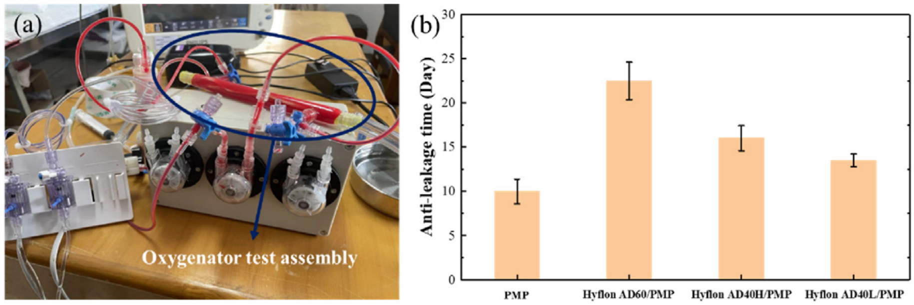

Fig. 6. (a) Oxygenator test assembly and (b) anti-leakage times of PMP hollow fiber membrane and three kinds of Hyflon AD/PMP composite membranes.

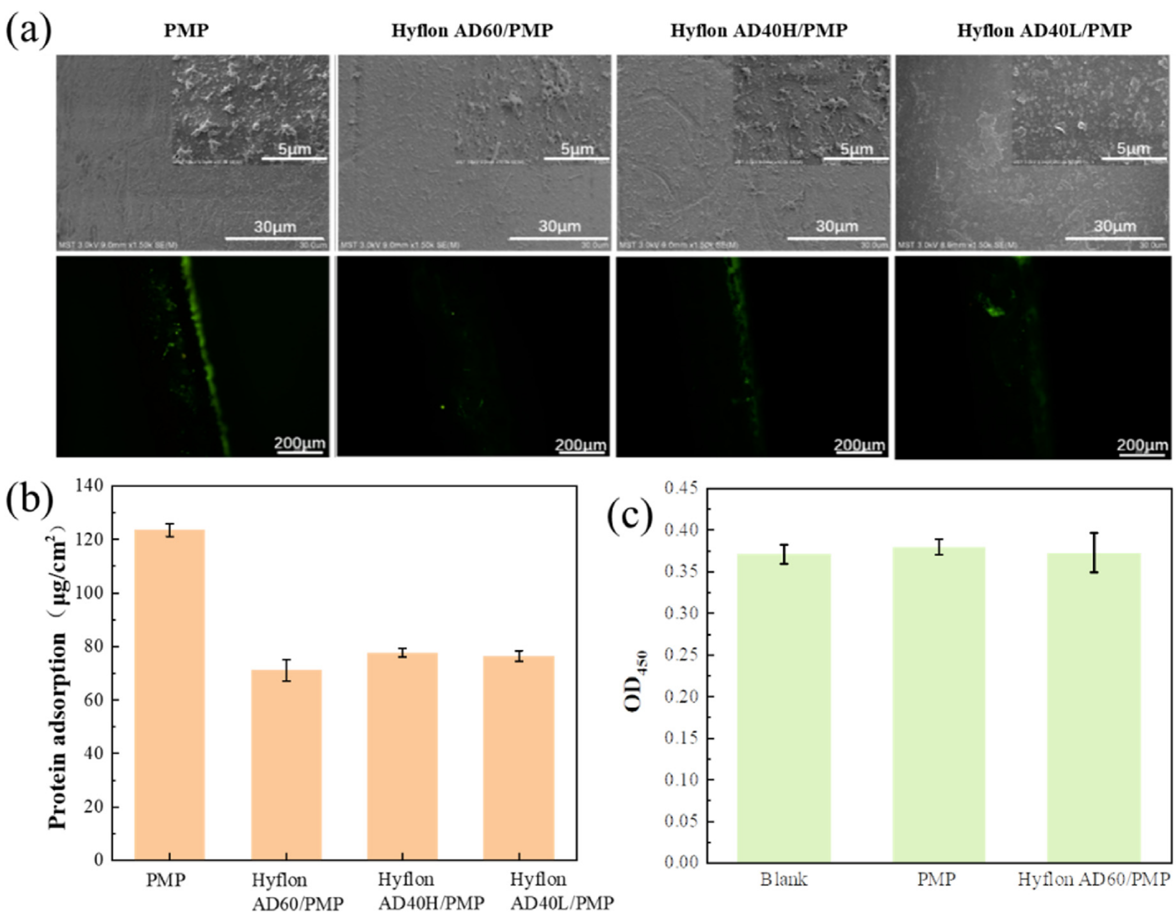

Fig. 7. (a) Platelet adhesion, (b) protein adsorption, and (c) cytotoxicity of the PMP hollow fiber membrane and three kinds of Hyflon AD/PMP hollow fiber composite membranes.

3.4. Evaluation of hemocompatibility performance

3.4.1. Platelet adhesion

Platelet adhesion electron microscopy and fluorescence characterization were performed on the pristine PMP membrane and three kinds of Hyflon AD/PMP composite membranes, as in Fig. 7a. Platelet adhesion of all Hyflon AD(40H, 40L and 60)/PMP hollow fiber composite membranes was lower than that of the pristine PMP membrane, consistent with the observed fluorescence intensity. A series of physiological rejection reactions (such as activation) occur when platelets adhere heavily to the surface of exogenous substances in the blood [41]. A membrane surface with good hemocompatibility should have very low platelet adhesion.

Table 4
Hemolysis rate of the PMP hollow fiber membrane and three kinds of Hyflon AD/PMP hollow fiber composite membranes.

Type	PMP	Hyflon AD60/PMP	Hyflon AD40H/PMP	Hyflon AD40L/PMP
Hemolysis rate (%)	3.2 ± 0.6	2.4 ± 0.4	2.9 ± 0.5	2.6 ± 0.7

3.4.2. Hemolysis test

The blood not in contact with the material was set as a blank control. The absorbance was measured at 540 nm using an enzyme standard meter after blood contacted the surfaces of different materials, and the hemolysis rate of the material was calculated. As can be seen in Table 4,

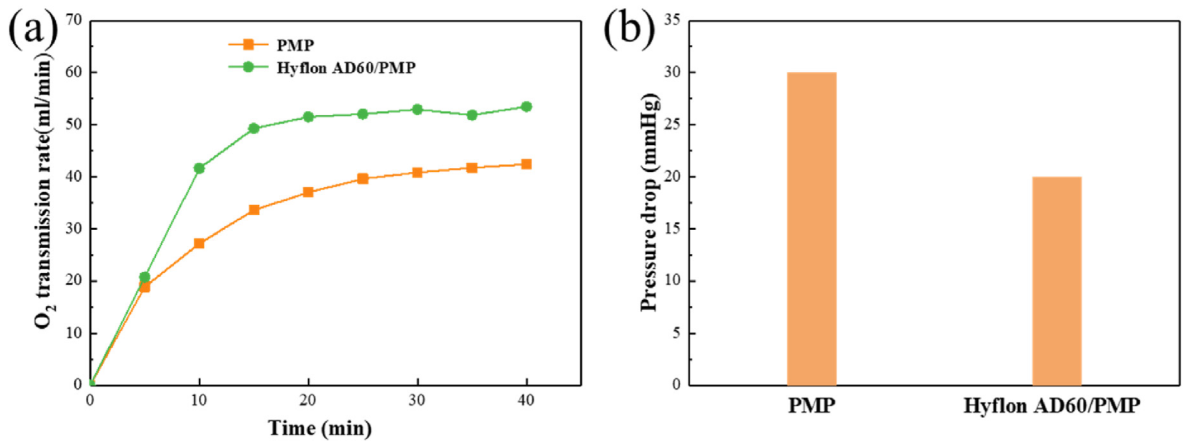

Fig. 8. Comparison of (a) oxygen exchange rate and (b) pressure decrease between the PMP hollow fiber membrane and Hyflon AD60/PMP hollow fiber composite membranes.

the hemolysis rate of the PMP membrane and Hyflon AD/PMP composite membrane met the Chinese national standard (ISO 10993 and GB/T16886) of less than 5%, indicating that the Hyflon AD layer does not damage blood and has high biosafety, conforming to the medical standards.

3.4.3. Protein adsorption

The static adsorption test of protein at 0.5 g/L was conducted on a homemade PMP hollow fiber membrane and Hyflon AD (40H, 40L, and 60)/PMP hollow fiber composite membranes. The corresponding results exhibited reduced adhesion of protein with all Hyflon AD membrane coatings. The protein adhesion value was reduced from 120 μg/cm^2 for the pristine PMP membrane to 70 μg/cm^2 for the Hyflon AD60 membrane, as shown in Fig. 7b. The mechanism of reduction of protein adsorption on the Hyflon AD membranes was the low surface free energy of the C–F bonds, which provided a double hydrophobic rather than hydrophilic or lipophilic surface.

3.4.4. Cytotoxicity test

We tested the absorbance at 450 nm after 24 h of cell culture and calculated the in vitro cell survival rate using Eq. (4). In that equation, A_s is the absorbance of experimental material, A_b is that of the blank sample, and A_c is the that of the control sample. Calculation of A_s-A_b is the absorbance of the PMP membrane or the Hyflon AD60/PMP composite membrane, and A_c-A_b is absorbance of the blank control group:

$$\text{Cell survival rate} = [(A_s - A_b) / (A_c - A_b)]*100\% \tag{4}$$

As shown in Fig. 7c, the cell survival rates of the pristine PMP membrane and the Hyflon AD60/PMP hollow fiber composite membranes were 100.54% and 102.34%, respectively, demonstrating that the adopted polymers were not cytotoxic and met the biosafety metrics of medical membrane materials.

3.5. Oxygenation performance of membranes

The oxygen exchange rate and pressure decrease of the PMP membrane and Hyflon AD60/PMP hollow fiber composite membrane were tested at a liquid flow rate of 300 mL/min and liquid-gas flow rate ratio (V/Q) of 5. As shown in Fig. 8a, the Hyflon AD60 membrane (50 mL/min) had a higher oxygen exchange rate than that of the pristine PMP membrane (40 mL/min). Additionally, the change in pressure decrease of the Hyflon AD60 membrane was smaller than that of the pristine PMP membrane, as in Fig. 8b, which implies that the Hyflon AD60/PMP hollow fiber composite membrane has higher pressure driving force and better oxygen exchange stability. That is, the smaller pressure decrease ensures better gas exchange efficiency during the gas separation process. The micropores on the surface of the pristine PMP hollow fiber membrane directly affected the ability to transmit oxygen through the membrane. This is because the micropores produce oxygen bubbles in the blood, affecting blood flow and increasing transport resistance. In this experiment, the effective membrane area of the homemade module was 0.3 m^2. The effective membrane area in actual use is about 1.8 m^2, producing an oxygen transmission rate of 300 mL/min, meeting the requirement for practical utilization (oxygen transmission rate ≥260 mL/min). Additionally, the stability of the composite membranes is crucial for future clinical applications. In the ECMO system, O_2 molecules permeate from upstream to downstream of the membrane module while CO_2 molecules permeate in the opposite direction. Therefore, traditional gas test devices can't directly evaluate the stability of the optimized Hyflon AD60/PMP composite membranes. We plan to adopt live animal experiments in the collaborator's laboratory to further evaluate the stability of the composite membrane, which will be reported in our near future work.

4. Conclusions

This work focused on preparation of Hyflon AD/PMP hollow fiber composite membranes to improve oxygenation and anti-leakage performance. As there were common and unavoidable defects on the surface of PMP membranes, they were hydrophobically modified via a perfluorinated Hyflon AD coating. When the concentration of Hyflon AD60 coating was 0.5 wt% and the coating time was 10 min, the optimized Hyflon AD60/PMP hollow fiber composite membrane exhibited appropriate gas performance and hemocompatibility suitable for ECMO systems in clinical use. The corresponding experimental results confirmed that the hydrophobic coating layer reduces protein and platelet adhesion and prolongs the time to leakage, ultimately achieving long-lasting PMP membranes. Therefore, the Hyflon/PMP hollow fiber composite membrane has potential in long-lasting application of ECMO and may improve clinical service life and oxygenation efficiency. Our modification method is convenient and suitable for further large-scale production.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their appreciation for the financial supported by the National Key R&D Program of China

(2020YFC0862903), the National Natural Science Foundation of China (22078146), the Key R&D program of Jiangsu Province (BE2021022), Jiangsu Province 100 Foreign Expert (BX2019106) and Cultivation Program for The Excellent Doctoral Dissertation of Nanjing Tech University.

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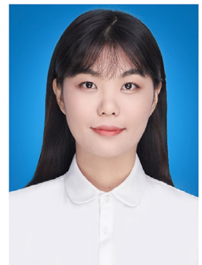
Prof. Young Moo Lee: Former President of Hanyang University, Korea; Fellow of the Korea Institute of Science and Technology; Editor of Journal of Membrane Science (since 2004). Mainly engaged in polymer structure design, new membrane material development and process research for gas separation, water treatment and fuel cell membrane. Published in Science (2 articles), Nature (1 article), Energy & Environmental Science, Chemical Reviews, and Progress in Polymer. He has published more than 380 papers in SCI Journals, obtained more than 150 patents, and published 5 monographs and 10 chapters. He has received more than 100 research MEDALS from the Korea research Foundation and more than 50 research MEDALS from the Korea Science and Engineering Foundation. He was awarded the first South Korean Government "Green Technology" Award in 2010, the South Korean "Knowledge Innovation" Award in 2010, and the South Korean "Kyung-Am" Academic Award in 2012. He has been the president of the Korea Film Association and the Asia Pacific Film Association. He was a visiting professor of Taiwan Central Yuan University (2010), an honorary professor of Harbin Institute of Technology (2011), an honorary professor of Jilin University (2015) and an honorary professor of Tianjin University (2017).

Dr. Zhaoliang Cui graduated from Nanjing Tech University with a doctorate in Engineering. From September 2011 to May 2014, he served as a postdoctoral and research assistant professor at Hanyang University in South Korea. In 2014, he returned to China and served as a professor in Nanjing University of Technology, established the organic/ultrafiltration membrane research group, and presided over projects such as the National Natural Science Foundation of China, the Natural Science Foundation of Jiangsu Province and the Launching Fund for the Return of Overseas Students of the Ministry of Education. His research interests include theoretical research and industrialization of polymer porous membranes for water treatment applications, and development of ECMO materials.



Ting He is currently pursuing her PhD in Nanjing Tech University. Previous work has mainly focused on surface modification of materials to regulate the growth of microorganisms on the surface of materials. Her research focuses on the preparation and modification of ECMO special hollow fiber membrane, and the mass transfer study of gas-blood exchange membrane.