Preparation of Temperature-Sensitive Hollow Fiber Membrane and Its Detection as a Cell Carrier

Hao Wang, Yiyi Sun, Jingwen Wang, Yuhuang Yuan, Yuen Yee Cheng, Xiangqin Li, and Kedong Song

Abstract NIPAAm has been widely used in cell culture and non-destructive desorption in recent years because it can change its surface configuration by changing temperature. In this study, NIPAAm was grafted onto the surface of the HFMs with cerium ammonium nitrate (CAN) as the initiator to prepare the thermosensitive HFMs. This material was used as a cell carrier for temperature sensitivity and biocompatibility assays to achieve non-destructive harvesting of cells from the surface of the HFMs. The morphology showed that poly (n-isopropylacrylamide) (PNIPAAm) was successfully grafted onto the HFMs. The results of water contact angle at different temperatures showed that the prepared samples have good temperature sensitivity. At 37 °C, PC12 cells adhered to and diffused well on the surface of PNIPAAm-g-HFMs. After cooling to 20 °C, the cells fell off the surface of the membrane in sheet form and retain more extracellular matrix proteins. PNIPAAm-g-HFMs in this study have good biocompatibility and can be better used as cell carriers in cell culture in vitro.

Keywords Temperature-sensitive · Hollow fiber membrane · Cell carrier · Biocompatibility · NIPAAm

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1 Introduction

Medical fields such as tissue engineering and cell therapy require a large number of cells that maintain the original biological characteristics. In the process of human stem cell therapy, the amount of cells required for clinical treatment is about $1.5 \sim 3 \times 10^6$ cells/kg [1], and the number of seed cells that can be derived from animal body or tissue is limited and far from meeting experimental and clinical treatment needs. Therefore, large-scale culturing and harvesting of cells in vitro is of great significance in the field of regenerative medicine. Due to its large specific surface area, the HFMs provide a large adhesion area for cells in a relatively small volume. In addition, due to the special porous wall structure of HFMs, cells can be cultured on its surface. The faster supplementation by nutrients such as glucose and the discharge of metabolic wastes such as lactic acid promote the proliferation of cells on the surface of HFMs [2]. Therefore, using HFMs as a cell carrier for the culture and harvesting of cells without enzymatic hydrolysis is a favorable way to achieve large-scale cell expansion.

Currently, the novel cell harvesting methods are mainly focused on stimuli-responsive polymer mediation [3, 4]. Examples include temperature-responsive polymers [5, 6], pH-responsive polymers [7, 8], light-responsive polymers [9, 10] and magnetic field-responsive polymers [11, 12], etc. PNIPAAm is a common temperature-responsive polymer that has been extensively studied in cell culture and achieves non-destructive desorption via its critical solution temperature (LCST, 32 °C) [13, 14] which is more consistent with the temperature of cell culture.

In this paper (as shown in Fig. 1), we used cerium ammonium nitrate (CAN) as an initiator to graft PNIPAAm to lignocellulose HFMs by free radical polymerization for preparation of thermosensitive HFMs for better cell adhesion, cultivation and cooling desorption. It provides a new idea for large-scale cell culture in vitro.
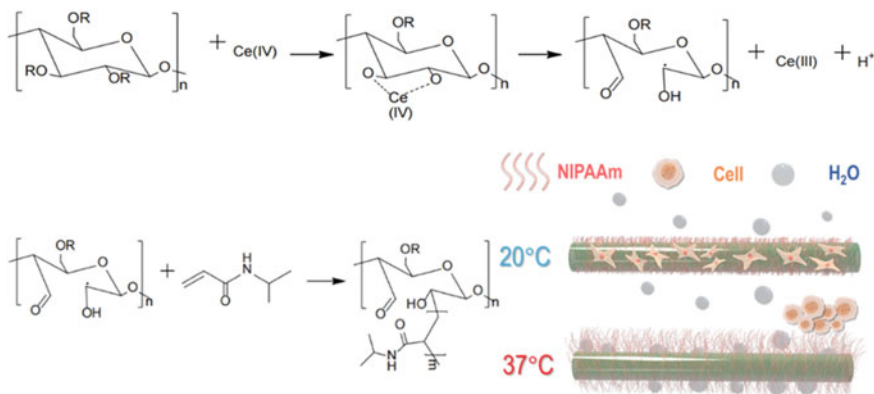
Fig. 1 Schematic illustration of the study design: preparation of PNIPAAm-g-HFMs and its application in cell culture

2 Materials and Methods

2.1 Preparation of PNIPAAm-Grafted-HFMs

Recrystallization of NIPAAm: dissolve the purchased commercialized 5 g of NIPAAm in 50 mL of n-hexane, fully dissolve it at 50 °C, then filter while hot, and collect the filtrate in a beaker. Place the filtrate at 4 °C overnight, filter and collect the filter cake, freeze dry the NIPAAm monomer with a freeze dryer for 24 h, and store the dried NIPAAm monomer in a sealed and dark place.

HFMs pretreatment: soak the lignocellulosic hollow fiber membrane in ethanol for 2 h, wash it with deionized water three times, and dry it in a vacuum drying oven at 25 °C. Soak the dried fiber membrane in 0.1 M NaOH solution at 60 °C for 24 h, and then dry it in a vacuum drying oven for standby.

PNIPAAm was grafted by solution free radical polymerization as follows [15]: the total length of 250 cm HFMs was placed in a three-port flask, 30 mL and 0.1 M HNO_3 solution was added and sealed with nitrogen bubble deoxidizing for 10 min, and heated to 60 °C in a water bath. Add the initiator ammonium ceric nitrate to make the final concentration to 10 mM. After activating for 45 min, cool the reaction system to 25 °C quickly, added NIPAAm monomer and reacted for 7 h under the protection of nitrogen (the reaction concentrations of NIPAAm monomers in the system were 0.01 M), and the final PNIPAAm-grafted-HFMs was recorded as HFMs-0.01. The HFMs that treated by NaOH alkali and marked as HFMs-0. At the end of the reaction, the HFMs-grafted-PNIPAAm was washed with a large amount of ddH_2O, and then the HFMs was sterilized by high pressure steam.

2.2 Morphology of PNIPAAm-Grafted-HFMs

The HFMs were brittle broken with liquid nitrogen, the surface was sprayed with gold, and the surface morphology of the HFMs was observed with a thermal emission environment scanning electron microscope. The test conditions were voltage 20 kV, current 10 mA, and 100× and 400× electron microscope images were taken respectively.

2.3 Water Contact Angle Test of PNIPAAm-Grafted-HFMs

Arrange the HFMs closely on the glass slide and place on the stage, adjust the position of the glass slide so that the sample is directly under the needle, set up the automatic contact angle test to measure the water contact angle by using a contact angle measuring instrument (DSA30S, Germany). More than 3 sets of independent

experiments were carried out for each group of samples, and the average value of all data was collected as the experimental result.

2.4 In Vitro Cell Culture on PNIPAAm-Grafted-HFMs

PNIPAAm-grafted-HFMs was laid on a 24-well cell culture plate, and the cell suspension of 50 μL, 8×10^5 cells/mL was slowly added and incubated at 5% CO_2 and 37 °C. In order to evaluate the viability of the adherent cells on the surface of HFM-0.01, the cells were stained with Calcein-AM/PI after 3, 5 and 7 days. Viable and dead cells were imaged using a fluorescence microscope (OLYMPUSBX71, Japan). The cellular HFMs complex fused to 80% on the temperature-sensitive HFM was removed from the old medium and introduced into a cold fresh serum-free medium (less than 20 °C). The cell-HFMs complex was incubated at 20 °C for 60 min, and the cell morphology was monitored by inverted phase contrast microscope.

2.5 Cell Desorption and Protein Content Detection

Cells fused to 90% on the temperature-sensitive HFMs were cooled and incubated until the cells became round, the HFMs was gently blown to collect the desorbed cells, and the undesorbed cells were harvested by trypsin digestion. The desorbed cells and undesorbed cells in HFMs-0.01 group were counted by blood cell counter, and the cell desorption rate was calculated as follows:

$$\text{detachment ratio} = \frac{n_1}{n_1 + n_2} \times 100\%$$

n_1: desorbed cells; n_2: undesorbed cells.

Detecting the protein content of cells harvested by trypsin digestion and cells of desorbing from HFMs-0.01, determining the effect of temperature sensitive desorption on the ability of cells to secrete proteins. The specific procedures are as follows, the total protein, fibronectin and laminin uploaded by PC12 cells were quantitatively evaluated and analyzed by BCA protein detection kit, fibronectin and laminin ELISA kit (Severn Innovation (Beijing) Biotechnology Co., Ltd.). Cells harvested from the HFMs-0.01 and trypsin digestion group were washed with cold PBS for 2–3 times, after centrifugation, added the prepared lysate (RIPA lysate 500 μL and PMSF 5 μL, mixed well, kept on ice for 5 min), and kept on ice for 30 min, centrifuged at 4 °C at high speed (12,000 rpm, 10 min), collected the supernatant and aliquoted, and stored it in a freezer at − 20 °C in the dark. Subsequently quantification of total protein, FN, and LN was performed according to the manufacturer's protocol to calculate the protein loading of individual cells.

2.6 Statistical Analysis

All data were obtained from at least three independent experiments. All data were statistically analyzed using Origin (2022, Origin Lab Corporation, USA). $* p < 0.05$ was considered statistically significant.

3 Results and Discussion

3.1 Surface Chemical Structure and Morphology of PNIPAAm-Grafted-HFMs

The morphology of HFMs-0 and PNIPAAm-grafted-HFMs were analyzed by SEM images before and after copolymerization (as shown in Fig. 2). Pristine HFMs exhibited smoother surface topography. When PNIPAAm was grafted and polymerized, there are a large number of uneven hillock-like protrusions appeared on the surface of HFMs, and the roughness increased. The result can prove that NIPAAm has been successfully grafted onto the surface of the fiber membrane.
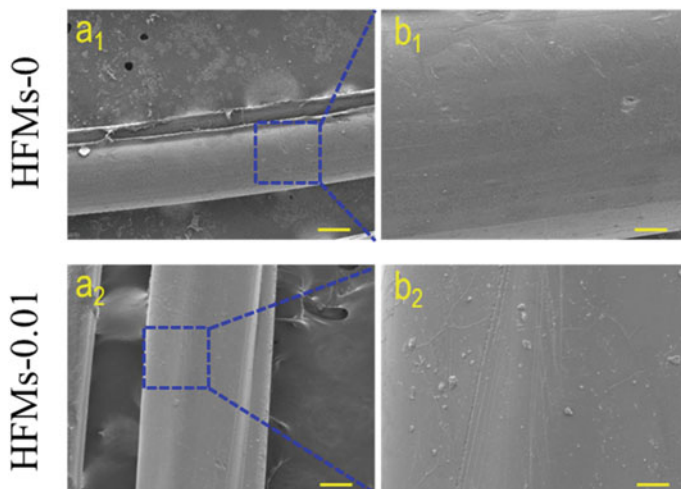
Fig. 2 Characterization of the overall structure of HFMs-0 and HFMs-0.01. SEM images of HFMs surface (a_1 SEM images of HFMs-0, a_2 SEM images of HFMs-0.01, scale bars: 100 μm, and b_1–b_2 is a local magnification of a_1–a_2, scale bars: 25 μm)

Fig. 3 Hydrophilic and
hydrophobic characterization
of HFMs-0 and HFMs-0.01.
The water contact angles of
different HFMs at 37 and
20 °C were measured by
liquid drop method
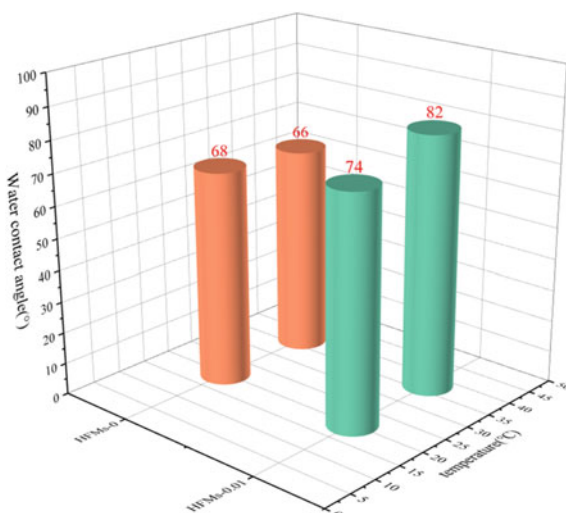
## 3.2 Temperature Sensitive Behavior of PNIPAAm-Grafted-HFMs

The water contact angles of HFMs-0 and temperature-sensitive HFMs-0.01 can be studied macroscopically by using a contact angle measuring instrument (as shown in Fig. 3). The fiber membranes of each group showed different water contact angles at different temperatures, indicating good temperature-sensitive properties of the HFMs.

3.3 Cell Culture on the Surface of HFMs

As shown in Fig. 4, we found that PC12 cells adhered, spread and proliferate normally on HFMs-0.01, and the fluorescent staining shows that the cells can survive normally on the surface of the fibrous membrane.

3.4 Cell Desorption Efficiency and Quantitative Determination of Protein

The detachment rate of adherent cells on HFMs-0 and HFMs-0.01 were measured as shown in Fig. 5a. The desorption rate of cells in the HFMs-0 group without PNIPAAm graft modification was $4.47 \pm 1.29\%$. HFMs-0.01 showed the desorption at $92.48 \pm$
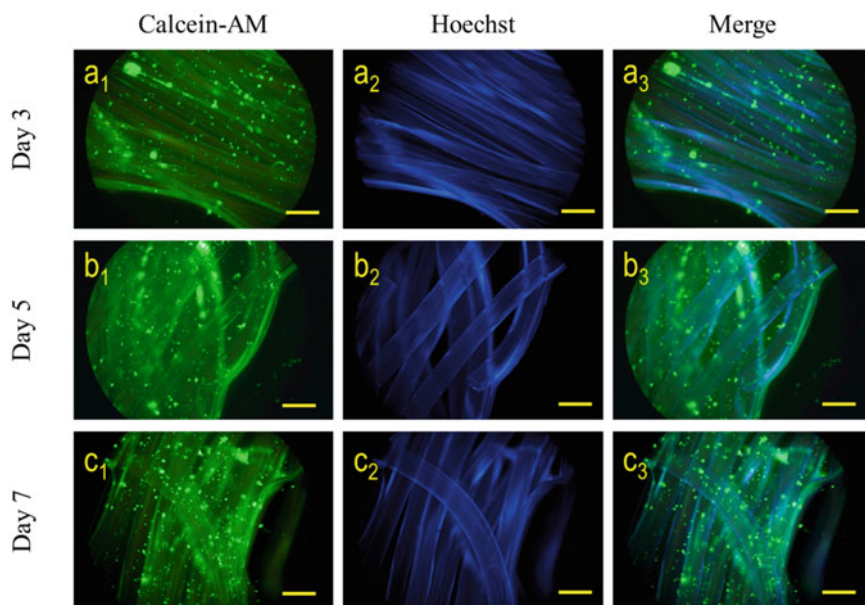
Fig. 4 Fluorescence microscopic image of PC12 cells cultured with HFMs-0.01. a_1–a_3 Fluorescence image of PC12 cells cultured on HFMs for 3 days. b_1–b_3 Fluorescence image of PC12 cells cultured on HFMs for 5 days. c_1–c_3 Fluorescence image of PC12 cells cultured on HFMs for 7 days. Scale bars: 200 µm

2.7%. The results showed that the cells cultured on the surface of HFMs-0.01 in this study could effectively desorb.

Due to the good temperature response characteristics of PNIPAAm-grafted-HFMs, the cells cultured on PNIPAAm-grafted-HFMs surface can be separated without trypsin treatment by reducing the temperature from 37 °C (hydrophobic) to 20 °C (hydrophilic). Figure 5b showed that under the static temperature of 20 °C, after 60 min, almost all the cells on the surface of HFMs-0.01 become round, thus falling off from the surface of the fibrous membrane.

Quantitative detection was performed on the cells obtained by the temperature-sensitive cooling desorption and the trypsin digestion methods (Table 1). In comparison of the two methods for multiple passages, the total protein, fibronectin and laminin loadings of the cells, the temperature-sensitive cooling desorption group showed higher than those in the trypsinization group, which further confirmed that cells harvested by temperature-sensitive cooling desorption can retain more extracellular matrix proteins and maintain higher cell proliferation activity.
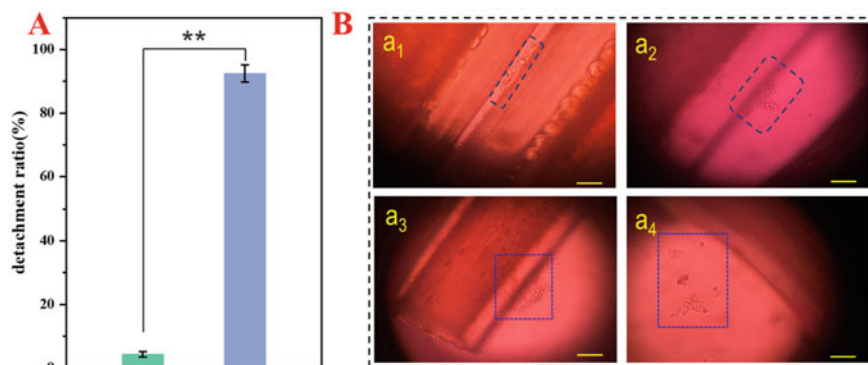
Fig. 5 **a** Detachment ratio of PC12 cells from HFMs-0 and HFMs-0.01 with temperature reduction (** $p < 0.01$; n = 3). **b** The time-course of morphology change of PC12 cells on the surface of HFMs-0.01 after reducing temperature from 37 to 20 °C. a_1 is the cell morphology after 15 min of reducing temperature. a_2 was the cell morphology after 30 min of reducing temperature. a_3 is the cell morphology after 45 min of reducing temperature. a_4 is the cell morphology after 60 min of reducing temperature. Scale bars: 100 μm

Table 1 Total protein, laminin and fibronectin and of PC12 cells obtained by different harvesting methods

Proteins	Thermal-liftoff groups	Trypsinization groups
Total protein (ng/cell)	0.0511 ± 0.0003	0.0142 ± 0.0002
LN (pg/cell)	0.0531 ± 0.0032	0.0451 ± 0.0063
FN (pg/cell)	2.5045 ± 0.0001	0.6709 ± 0.0043

4 Conclusion

In this study, PNIPAAm-grafted-HFMs were prepared by free radical polymerization. The SEM test showed that NIPAAm was successfully grafted onto the surface of the fiber membrane. The surface of HFMs showed different hydrophilic and hydrophobic characteristics with different grafting concentrations. The group with the largest difference in contact angle was selected for cell desorption experiment, and the cells could effectively peel off from the substrate surface within 60 min. Cells harvested by the cooling method can retain more extracellular matrix proteins and maintain higher cell activity. The above results show that the temperature sensitive substrate can be used as a cell carrier to effectively culture and expand cells in vitro.

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