·论　著·

Research on the glucose-responsive gating membranes for controlled insulin delivery

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ABSTRACT: The pore size and permeability control of a glucose-responsive gating membrane with plasma-grafted poly(acrylic acid) (PAAC) gates and covalently bound glucose oxidase (GOD) enzymes were investigated systematically. The PAAC-grafted porous polyvinylidene fluoride (PVDF) membranes with a wide range of grafting yields were prepared using a plasma-graft pore-filling polymerization method, and the immobilization of GOD was carried out by a carbodiimide method. The linear grafted PAAC chains in the membrane pores acted as the pH-responsive gates or actuators. The immobilized GOD acted as the glucose sensor and catalyzer; it was sensitive to glucose and catalyzed the glucose conversion to gluconic acid. The experimental results showed that, the glucose-responsivity of the solute diffusional permeability through the proposed membranes was heavily dependent on the PAAC grafting yield, because the pH-responsive change of pore size governed the glucose-responsive diffusional permeability. It is very important to design a proper grafting yield for obtaining an ideal gating response. For the proposed gating membrane with a PAAC grafting yield of 1.55%, the insulin permeation coefficient after the glucose addition (0.2 mol/L) was about 9.37 times that in the absence of glucose, presenting an exciting result on glucose-sensitive self-regulated insulin permeation.

KEY WORDS: Glucose-responsive; Insulin release; Intelligent drug delivery system; Gating membrane; Gating characteristics

葡萄糖浓度感应型胰岛素控制释放膜研究

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摘要: 葡萄糖感应型智能化胰岛素释放系统的研究正受到越来越广泛的重视和关注。本文系统研究了具有等离子体接枝聚丙烯酸高分子链和固定葡萄糖氧化酶的开关膜的响应特性，接枝在多孔聚偏氟乙烯膜孔上的聚丙烯酸链起到 pH 感应开关的作用，固定的葡萄糖氧化酶起到葡萄糖传感器和将葡萄糖转化为葡萄糖酸的催化剂作用。研究结果表明，该开关膜对葡萄糖的感应性极大程度地依赖于聚丙烯酸的接枝率，因为膜孔的 pH 响应性决定了其葡萄糖感应控释特性。因此，为了获得良好的葡萄糖感应开关特性，聚丙烯酸的接枝率必须控制在一个适当的范围。对于聚丙烯酸接枝率为 1.55% 的开关膜，当外界葡萄糖浓度从 0 上升到 0.2 mol/L 时，胰岛素的扩散透过系数猛然增大了 9.37 倍，显示出了良好的葡萄糖感应型自调节式胰岛素释放特性。

关键词: 葡萄糖感应; 胰岛素释放; 智能化给药系统; 开关膜; 开关特性

Introduction

The development of a glucose-sensitive insulin-releasing system for diabetes therapy is a long-standing challenging for biomedical engineers[1,2]. Although diabetes mellitus is a major cause of death in industrialized countries, periodical parenteral injections of insulin are currently the standard treatment for insulin-dependent diabetic patients. However, poor control of blood glucose level and poor patient compliance are associated with this method[3]. Therefore, there is a need for self-regulated delivery systems having the capability of adapting the insulin release rate in response to changes in glucose concentration in order to keep the blood glucose levels within the normal range[4].

Up to now, several kinds of glucose-responsive insulin delivery systems have been devised[3~7]. However, none of these systems could fully mimic the physiology of insulin secretion as yet[4]. Therefore, better ways of glucose-responsive self-regulated administration of insulin delivery are still being sought. For glucose-responsive self-regulated insulin release systems, stabili-

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ty and responsivity of the system are very important and essential, because only stable system can ensure the safety during therapy and only fast response can ensure the self-regulated insulin-release exactly during changes in glucose concentration. To meet both stability and responsivity, glucose-responsive gating membranes with porous substrates and linear-grafted functional polymeric gates are competent. The porous membrane substrates can provide mechanical strength and dimensional stability. As the linear grafted polymeric chains have freely mobile ends, which are different from the typical crosslinked network structure of the hydrogels that gives rise to relatively immobile chain ends, the responsiveness of the prepared membranes to the environmental stimuli could therefore be faster than that of their corresponding homogeneous analogs, owing to the more rapid conformational changes of the functional polymers. By grafting pH-responsive polymeric chains onto porous membrane substrates and immobilizing glucose oxidase (GOD) onto the grafted polymers, glucose-responsive gating membranes have been prepared for insulin release[5,6], and the response time of the membrane to glucose was reported as fast as 16 s or even shorter[6]. Unfortunately, although some relevant investigations have been carried out[5,6], a comprehensive understanding of the gating characteristics of this kind of glucose-responsive membranes, including that of the control of the membrane pore size and the permeability response, is still lacking. In this study, glucose-responsive gating membranes with grafted poly(acrylic acid) (PAAC) gates and covalently bound glucose oxidase (GOD), were prepared by grafting PAAC onto porous polyvinylidene fluoride (PVDF) membrane substrates with a plasma-graft pore-filling polymerization method[8~11], and immobilizing GOD onto the grafted membranes with a carbodiimide method[5]. The preparation process route and the principle of glucose-responsive control of the permeation through the gating membrane are schematically illustrated in Fig. 1. Investigations were carried out on the morphological examination of the grafting, on the control of pH-responsive pore size of the membrane and pH- and glucose-responsive permeability through the membrane by the PAAC grafting yield, and on the glucose-responsive insulin diffusion coefficient.

1 Materials and methods

1.1 Materials

Porous polyvinylidene fluoride (PVDF) membranes were used as the porous membrane substrates. The PVDF substrate, with a pore size of 0.22 μm and a thickness of 62.5 μm, was supplied by Hangzhou Xidoumen Membrane Co. Ltd, China. Bovine pancreas insulin (28.5 USP units/mg) was purchased from Sigma Chemical Co., and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride was obtained from Acros Organics Co., USA. Glucose oxidase (GOD, 127 U/mg) was purchased from Toyobo Co., Ltd., Japan. 2-(N-morpholino) ethanesulfonic acid (MES) was obtained from Shanghai Yuanju Biotech Co., Ltd., China. Glucose, acrylic acid (AAC) and sodium chloride were purchased from Chengdu Kelong Chemical Reagent Co., Ltd, China. The water used in the experiments was well-deionized and whose resistance was larger than 16 MΩ.

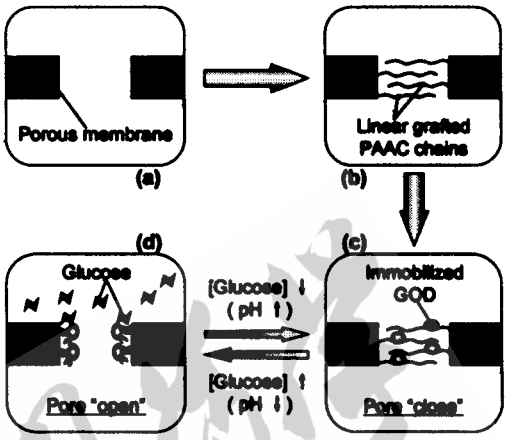

Fig 1 Schematic illustration of the preparation process route and the principle of glucose-responsive control of the permeation through the gating membrane

1.2 Grafting poly(acrylic acid) gates by plasma-graft pore-filling polymerization

Plasma-graft pore-filling polymerization was employed to graft the linear poly(acrylic acid) (PAAC) chains into the pores of the PVDF membrane substrate according to the method described previously[8~11]. In the experiments, the AAC concentrations in the monomer solutions were from 3 wt.% to 7 wt.%, and the grafting time was from 60 min to 300 min. The PAAC-grafted membranes were washed three times with deionized water under vibration in a constant-temperature bath (30℃) for 24 h to remove any non-reacted monomer and homopolymer. It was then dried in a vacuum oven at 50 ℃.

The grafting yield of PAAC onto the PVDF membrane was defined as the weight increase of the membrane after the grafting, and could be calculated according to the following equation:

$$Y = \frac{W_g - W_o}{W_o} \times 100\% \qquad (1)$$

where, Y stands for the grafting yield of PAAC onto the membrane substrate [%], and Wg and W0 for the mass of the membrane after and before grafting respectively [g].

1.3 Preparation of glucose-responsive membranes by immobilizing glucose oxidase

Immobilization of glucose oxidase was carried out by the carbodiimide method that was described by Ito et al[5].

1.4 FT-IR analyses of membranes

Fourier transform infrared (FT-IR) spectra of the ungrafted and the PAAC-grafted membranes were measured on a spectrophotometer (Spectrum one, P-E Com., USA), to ascertain the

grafted PAAC formation.

1.5 Estimation of pH-responsive pore size change by filtration experiments

The pH-responsive changes of pore size of membranes with different grafting yield of PAAC were estimated by filtration experiments on aqueous solutions. The hydraulic permeability experiments or filtration experiments of membranes were carried out with trans-membrane pressure being 90 kPa. The diameter of the effective membrane area for filtration was 60 mm. The temperature of the feed solutions, buffered at pH 4 and pH 7 respectively, was controlled at 30℃ using a thermostatic unit (DC-0506, Shanghai Hengping Instrument Co., China). The hydraulic permeability through the ungrafted and the PAAC-grafted membranes under different pH conditions was studied by measuring the water flux. To minimize the experimental errors, the flux measurements were carried out three to five times and the arithmetically averaged values were taken as the results under each condition. The pH-responsive change of pore size of PAAC-g-PVDF membranes could then be calculated according to Hagen-Poiseuille's law.

1.6 Glucose-responsive diffusion experiments

The diffusional permeability experiments of membranes with grafted PAAC and immobilized GOD were carried out using a standard side-by-side diffusion cell. The diffusion cell was located in a constant – temperature water – bath to keep the diffusional temperature constant (30℃), and the solutions in both the donor and the receptor compartments were magnetically stirred. Each test membrane was immersed in the permeant solution overnight before starting the diffusion experiments. When the solute was sodium chloride, the initial NaCl concentration in the donor side was 0.2 mol/L and well-deionized water was used as the liquid in the receptor cell. The concentration increase of NaCl in the receptor was determined by measuring the electrical conductance with an electrical conductivity meter (DDS-390, Chengdu Fangzhou Science and Technology Co., China). When insulin was used as the solute, a 0.1 mol/L Tris-HCl aqueous solution was used as the buffer solution and the initial insulin concentration in the donor side was 0.1 mg/mL, and pure 0.1 mol/L Tris-HCl-buffered solution was used in the receptor compartment. The concentration increase of insulin in the receptor was measured using a UV-visible recording spectrophotometer (752W, Shanghai Analytical Instrument, China) at wavelength of $\lambda = 274$ nm.

The diffusion coefficient of solute across the membrane can be calculated using the following equation, derived from Fick's first law of diffusion [11]:

$$D = \frac{V_1 V_2}{(V_1 + V_2)} \cdot \frac{L}{A} \cdot \frac{1}{t} \cdot \ln \frac{C_f - C_i}{C_f - C_t} \qquad (2)$$

where D is the diffusional coefficient, [cm^2/s]; C_i, C_t, and Cf are the initial, intermediary (at time t), and final concentrations of solute in the receptor side respectively, [mol/L]; V_1 and V_2 are respectively the volume of the liquid in the donor compartment and that in the receptor compartment, [cm^3]; L is the thickness of the dry membrane, [cm]; and A is the effective diffusion area of the membrane, [cm^2].

In all the experiments, a plot of $\ln[(C_f - C_i)/(C_f - C_t)]$ (from Equation (2)) against time, t, showed a straight line. From this, the permeability coefficient could be calculated using the following equation:

$$D = K \cdot \frac{V_1 V_2}{(V_1 + V_2)} \cdot \frac{L}{A} \qquad (3)$$

where, K is the gradient of the line from the $\ln[(C_f - C_i)/(C_f - C_t)]$ versus t plots.

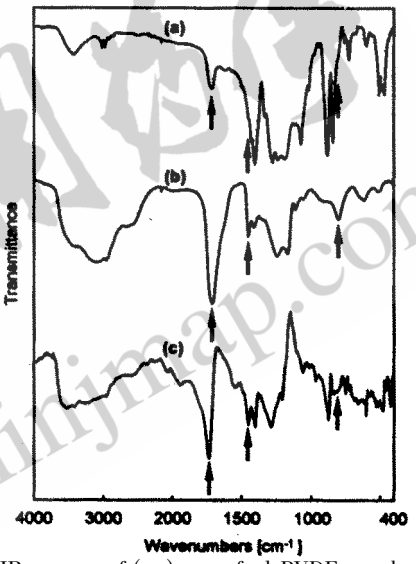

Fig2 FT-IR spectra of (a) ungrafted PVDF membranes; (b) PAAC; and (c) PAAC-g-PVDF membranes

2 Results and discussion

2.1 FT-IR analyses of the PAAC-grafted membranes

FT-IR spectra of ungrafted and PAAC-grafted PVDF membranes are illustrated in Fig. 2. After grafting PAAC onto the PVDF porous substrate, the peak at 1715 cm^{-1} (which is the characteristic peak of PAAC) was enhanced, and two other characteristic peaks of PAAC at 1453 cm^{-1} and 800 cm^{-1}, appeared newly in the spectrum comparing with that of the ungrafted membrane. The comparison result confirmed that PAAC was grafted on the membrane substrate by plasma-graft pore-filling polymerization.

2.2 Estimation of pH-responsive control of the pore size of PAAC-grafted membrane

The pH-responsive change of pore size of PAAC-g-PVDF membranes can be estimated using Hagen-Poiseuille's law. According to Hagen-Poiseuille's law, the water flux of a skinless porous membrane can be expressed as:

$$J = \frac{n\pi d^4 P}{128\eta\iota} \quad (4)$$

where, J stands for the water flux, [$m^3 \cdot m^{-2} \cdot s^{-1}$]; n for the number of pores per unit area, [m^{-2}]; d for the pore diameter, [m]; P for the trans-membrane pressure, [Pa]; η for the viscosity of flowing liquid, [Pa · s]; and l for the membrane thickness, [m].

In a plasma-graft pore-filling membrane, the grafted polymer forms a skin layer on the inner surface of the membrane pore[11]. As indicated in Equation (4), the water flux is governed by the fourth power of the pore diameter. Thus the conformational change of PAAC chains that grafted on the inner surface of membrane pore, i. e., coil-globule, affects the water flux greatly. As known from Equation (4), the ratio of effective pore diameter of the PAAC-grafted membrane at pH = 4 to that at pH = 7, which is defined as the pH-responsive gating factor of the membrane pore size, can be calculated using the measured water fluxes and viscosities of water under different pH conditions with the following equation:

$$N_{pH=4/pH=7} = \frac{d_{g,pH=4}}{d_{g,pH=7}} = \left(\frac{J_{pH=4}\eta_{pH=4}}{J_{pH=7}\eta_{pH=7}}\right)^{1/4} \quad (5)$$

where, NpH = 4/pH = 7 is the pH-responsive gating factor of the membrane pore size; dg, pH = 4 and dg, pH = 7 are the effective pore diameters of the PAAC-grafted membranes at pH = 4 and pH = 7 respectively, [m]; JpH = 4 and JpH = 7 are respectively the measured water fluxes through PAAC-grafted membranes at the environmental pH = 4 and pH = 7, [$m^3 \cdot m^{-2} \cdot s^{-1}$]; and ηpH = 4 and ηpH = 7 are the viscosity coefficients of flowing liquid at pH = 4 and pH = 7 respectively, [Pa · s].

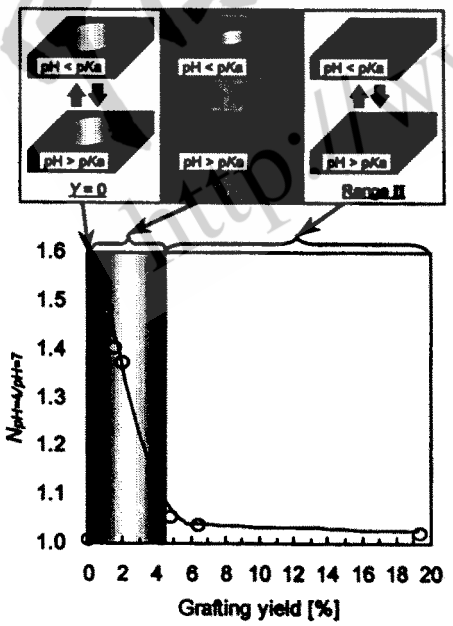

Fig 3 Effect of the grafting yield on the pH-responsive gating factor of membrane pore size and schematic illustration of the pH-responsive control of pore size

Figure 3 shows the effect of grafting yield on the pH-responsive gating factor of membrane pore size and the schematic illustration of pH-responsive control of pore size. For the membrane substrate, the pore size did not change with varying the environmental pH. For the PAAC-grafted membranes, the pH-responsive gating factor of membrane pore size was heavily affected by the grafting yield. When the grafting yield was less than 1.01 %, the pH-responsive gating factor of membrane pore size increased with increasing grafting yield. However, when the grafting yield is larger than 4.78 %, the pH-responsive gating factor of membrane pore size tended to 1. It can be seen that only when the grafting yield was less than 4.78 %, the grafted PAAC chains in the membrane pores acted as effective pH-responsive gates or adjusting valves. Whereas, when the grafting yield was larger than 4.78 %, the length and/or density of the grafted PAAC chains in the membrane pores were too long and/or large, resulting in that the chains lost the function of effective pH-responsive gates or adjusting valves.

When the grafting yield was too small, the grafted PAAC chains were too short, resulting in small responsivity of the membrane pore size. With increasing the grafting yield, the length and density of grafted PAAC chains increased, consequently the pH-responsive gating factor of membrane pore size also increased. However, when the grafting yield increased too large, the grafted PAAC chains becomes too long and/or too dense, and the conformational change of the PAAC chains could not bring any obvious change of the pore size any more. That is, the membrane pores had been "choked" by the grafted polymers. The influence behavior of the grafting yield on the flux responsiveness and that on the pH-responsive gating factor of membrane pore size were similar to each other. This verified again that the pH-responsive flux change of the PAAC-grafted membranes was governed by the pH-responsive gating factor of membrane pore size. For the environmental stimuli-responsive gating membrane, the larger the responsive gating factor of membrane pore size is, the better the gating property. Therefore, it is very important to design a proper grafting yield for obtaining an ideal gating response. In the present study, it was suggested to set the grafting yield in the range from 0.5 % to 3.0 % for the membrane to get a satisfactory pH-responsive gating property.

2.3 Glucose-responsive diffusional permeability

Figure 4 shows the effect of environmental glucose concentration on the diffusional permeation of NaCl through PAAC-grafted and GOD-immobilized PVDF gating membranes with different PAAC grafting yields. When the grafting yield was zero (i. e., the substrate membrane), the environmental glucose concentration nearly did not affect the diffusional permeability of solute molecules across the membrane. When the PAAC grafting yield

was 1.5 %, the diffusional permeability of NaCl molecules across the membrane was low in the absence of glucose; however it increased dramatically when the environmental glucose concentration was changed from 0 mol/L to 0.2 mol/L. On the other hand, when the grafting yield was as large as 7.5%, the diffusional permeability of NaCl solute across the membrane only increased a little with changing the environmental glucose concentration from 0 mol/L to 0.2 mol/L. From Fig. 4 it can be also found that the diffusional permeability of solute across the gating membrane generally decreases with increasing the PAAC grafting yield.

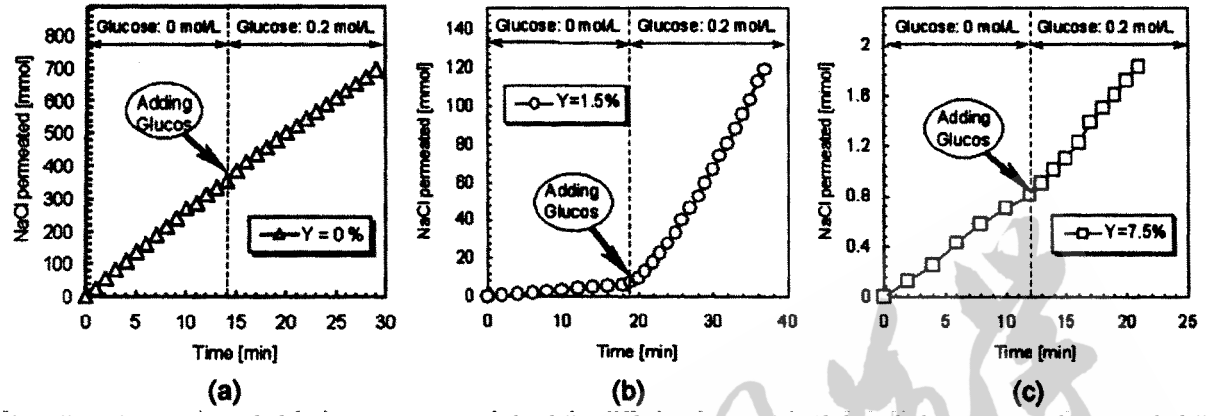

Fig 4 Effect of environmental glucose concentration on the diffusional permeation of NaCl through the gating membranes with different PAAC grafting yields: (a) substrate; (b) PAAC grafting yield is 1.5%; and (c) PAAC grafting yield is 7.5%

The prepared glucose-responsive gating membrane was composed of porous PVDF substrate, linear grafted PAAC chains in the pores and covalently bound GOD. The immobilized GOD acted as the glucose sensor and catalyzer; it was sensitive to glucose and catalyzed the glucose conversion to gluconic acid. Because of the appearance of gluconic acid, the local pH decreased in the microenvironment as a result. The linear grafted PAAC chains in the membrane pores acted as the pH-responsive gates or actuators. At neutral pH in the absence of glucose, the carboxyl groups of the grafted PAAC chains were dissociated and negatively charged, therefore the membrane gates "closed" because the repulsion between negative charges made the PAAC chains extended. On the other hand, when glucose concentration increased, GOD catalyzed the oxidation of glucose into gluconic acid, thereby lowering the local pH in the microenvironment, protonating the carboxylate groups of the grafted PAAC chains, therefore the gates "opened" because of the shrinkage of the chains resulted from the reduced electrostatic repulsion between the grafted PAAC chains in the pores. Because of the glucose-responsivity of the membrane pore size, the above-mentioned experimental phenomena about the glucose-responsive diffusional permeability of solute across the membrane were resulted. The results showed that the glucose-responsivity of the solute diffusional permeability was heavily dependent on the PAAC grafting yield, i.e., the above-mentioned pH-responsive change of pore size governed the glucose-responsive diffusional permeability.

Figure 5 shows the glucose-responsive diffusional permeability of insulin through the proposed gating membrane with PAAC grafting yield of 1.55% caused by glucose addition. In the absence of glucose, the diffusional permeation coefficient of insulin molecules across the membrane was as low as 0.79×10^{-7} cm^2/s, and the amount of insulin permeated increased linearly with time. When the environmental glucose concentration was changed from 0 mol/L to 0.2 mol/L by adding glucose, the insulin permeation coefficient increased to 7.40×10^{-7} cm^2/s dramatically. The permeation coefficient after the glucose addition was about 9.37 times that before the addition of glucose. The results presented an exciting glucose-sensitive self-regulated permeation of insulin molecules.

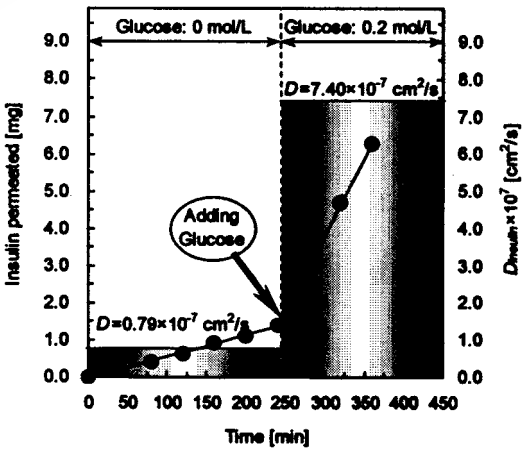

Fig 5 Glucose-responsive diffusional permeation of insulin through the proposed gating membrane with PAAC grafting yield of 1.55%

3 Conclusions

The glucose-responsivity of the solute diffusional permeability through the proposed membranes was heavily dependent on the PAAC grafting yield, because the pH-responsive change of pore

size governed the glucose-responsive diffusional permeability. It is very important to design a proper grafting yield for obtaining an ideal gating response. In the present study, it was suggested to set the grafting yield in the range from 0.5 % to 3.0 % for the membrane to achieve a satisfactory gating property. The experimental results showed an exciting glucose-sensitive self-regulated permeation of insulin molecules through the proposed gating membrane with a PAAC grafting yield of 1.55%, for which the insulin permeation coefficient after the glucose addition (0.2 mol/L) was about 9.37 times that before the addition of glucose.

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