Evaluation of asymmetric poly(vinyl alcohol) membranes for use in artificial islets

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Islets of Langerhans surrounded by a semipermeable membrane to prevent the host immunosystem is a potential way to treat type I diabetes mellitus. In this study, a series of poly (vinyl alcohol) membranes were formed by adding polyethylene glycols to create pores in the skin layer. The permeability study showed the skin layer structure had an influence on the diffusion of low molecular weight glucose, vitamin B12 and insulin. The mass transfer coefficient was improved from 1.04×10^{-4} to 2.16×10^{-4} cm/sec for glucose, from 2.84×10^{-5} to 8.36×10^{-5} cm/sec for vitamin B12 and from 1.45×10^{-6} to 4.15×10^{-6} cm/sec for insulin, whereas the passage of immunoglobulin G was completely prevented, indicating that these membranes could be effective in protecting islets from immunorejection. Thus such a membrane is an alternative potential material for artificial islets. In addition, we examined the insulin secretory response of islets separated by a poly(vinyl alcohol) membrane. We found that the insulin-secretion rate is relatively rapid compared to the permeation rate of insulin; thus, the process of the artificial islets is insulin-diffusion-controlled. © 1996 Elsevier Science Limited

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Since the development of an asymmetric type of membrane by Loeb and Sourirajan¹, the field of membrane science and technology has experienced an increasing growth. From the technological point of view, the most important properties of membranes are their functional properties such as permeability and permselectivity. For example, many techniques have been used to combine membrane technology with biological cells to treat a specific disease such as artificial pancreas^{2–11}. The membrane is permeable to small molecules such as glucose or insulin but totally impermeable to large molecules such as antibodies and lymphocytes. Therefore, it is necessary to prepare membranes of proper structure to meet the requirements of its application.

Reported publications on the artificial pancreas using commercially available membranes limited their performance. Therefore, the aim of this work was to synthesize a series of asymmetric poly(vinyl alcohol) membranes, evaluate their permeability to glucose, vitamin B12 and insulin and to examine whether they could prevent the passage of immunoglobulin G. Furthermore, we examined the insulin secretory response of islets separated by a poly(vinyl alcohol) membrane.

Asymmetric membranes are produced by the so-called 'phase inversion' technique¹². In this process, a casting solution consisting of polymer and solvent is immersed into a non-solvent coagulation bath. A solvent/non-solvent diffusional interchange causes the casting solution to bring about phase transition, which forms the membrane. It has been suggested that the membrane formation process can be considered as a two-step mechanical model¹³. The membrane is divided into two layers: top-layer (or skin layer) and sub-layer. With the casting solution immersed into the coagulation bath, the top-layer is formed first at the casting solution–non-solvent interface. Since solvent diffuses out very rapidly, the casting solution forms a dense skin layer. The dense layer will increase the barrier for diffusion of solvent from the sub-layer of casting solution. Therefore, when non-solvent diffuses into the sub-layer of casting solution, the composition of the sub-layer does not change and the sub-layer will proceed to liquid–liquid demixing to form a porous structure¹³.

MATERIALS AND METHODS

Membrane preparation

Poly(vinyl alcohol) was kindly supplied by Chang Chun Co. (Taiwan). The average molecular weight of

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poly(vinyl alcohol) used was about 74 000. Poly(vinyl alcohol) was dissolved in water to form a 14 wt% polymer solution at 90°C. A series of different molecular weight polyethylene glycols were added to 50 g of polymer solutions, as shown in *Table 1*. Polyethylene glycols #4000 and #2000 were purchased from Nacalai Tesque (Kyoto, Japan) with the average molecular weight being about 4000 and 2000, respectively. In poly(vinyl alcohol) membrane formation, polyethylene glycol was used as the pore former¹⁴ to increase the porosity and permeability of membranes.

The casting solution was kept at 25°C for 24 h and spread on a glass plate to a uniform thickness of about 100 µm by an autocoater, which was immediately immersed into a coagulation bath with Na₂SO₄/KOH/H₂O = saturate/75 g/1000 ml¹⁵ for 30 min to obtain a uniform membrane. The morphology of the membranes was examined using a scanning electron microscope (SEM).

Permeability measurements

The ability of poly(vinyl alcohol) membranes to allow diffusion of glucose, vitamin B12 and insulin (with a nominal activity of 28 IU/mg; Sigma) were studied at 37°C. Glucose and vitamin B12 concentration were characterized using a glucose analyser and spectrophotometer, respectively. Insulin concentration was determined by radioimmunoassay¹⁶. The permeabilities were measured using a two-chamber, well-stirred diffusion cell with a volume of 3.5 ml in each chamber. The membrane was placed between two chambers with 0.64 cm² of available membrane area. The stirring speed in each chamber was maintained at approximately 600 rpm using independently controlled motors. Since the permeability measurement was performed under sufficient stirring, the diffusion resistance at the liquid-membrane interface was neglected.

One of the diffusion chambers was filled with phosphate-buffered saline (PBS) (pH=7.4) containing the diffusion molecules and the other chamber was filled with PBS only. The diffusion of solutes through the membranes was monitored by periodically removing 50 µl samples from both chambers and an equal volume of solute-free PBS was refilled to avoid errors arising from volume variation. The mass balance can be written as

$$V \frac{dC_2}{dt} = \frac{A}{L} D(C_1 - C_2) \quad (1)$$

where C_1 and C_2 are concentrations of the solute in the donor and receptor chambers, respectively, A is the

exposed membrane area, L is the membrane thickness and V is the chamber volume. Equation (1) can be solved by analytical integration to give

$$\ln \left[\frac{(C_1 - C_2)_t}{(C_1 - C_2)_0} \right] = 2 \frac{DA}{LV} t \quad (2)$$

where $(C_1 - C_2)$ is the solute concentration difference at times 0 and t . Linear regression analysis of the above equation gave the slope $2DA/LV$, from which the mass transfer coefficient (D/L) was calculated. Each experiment was performed in duplicate and the results were expressed as the mean of the two results.

Islet isolation and *in vitro* test

An *in vitro* test was carried out to test the insulin secretory activity of islets prevented by the poly(vinyl alcohol) membrane. Islets were prepared from male Wistar rats (250–350 g) by a modification of the method of Lacy and Kostianovski¹⁷. The *in vitro* test used the same diffusion cell to evaluate the performance of poly(vinyl alcohol) membranes for the artificial islets. One of the diffusion chambers was filled with RPMI-1460 medium (pH=7.4) containing 500 mg/dl of glucose (glucose-side) and the other chamber was filled with glucose-free RPMI-1460 and about 200 rat islets of Langerhans (islet-side). The medium of both sides was sampled (125 µl) periodically to analyse the variation in concentration of glucose and insulin, and an equal volume of medium was refilled.

Immunoglobulin G (IgG) permeability test

An IgG permeability test used the same diffusion cell. One of the diffusion chambers was filled with PBS (pH=7.4), while the other chamber was filled with fresh serum. Blood from healthy donors was allowed to coagulate for 30 min at room temperature. After centrifugation, serum was collected. IgG was monitored by removing 100 µl samples until 100 h and was determined from fluorescence intensity.

RESULTS AND DISCUSSION

The permeation characteristics of the membranes are summarized in *Table 1*. The mass transfer coefficient in a given membrane decreased as the size of the diffusing molecules increased. *Figure 1* shows, for instance, the diffusion curves of glucose through membranes 1–4. It was seen that good linear dependence was obtained for other permeability

Table 1 Effect of pore former on mass transfer coefficients of glucose, vitamin B12 and insulin through poly(vinyl alcohol) membranes

Membrane no.	Pore former (g) (Polyethylene glycol)	Glucose (cm/sec) × 10 ⁻⁴	Vitamin B12 (cm/sec) × 10 ⁻⁵	Insulin (cm/sec) × 10 ⁻⁶
1	0	1.06	2.84	1.45
2	0.5 (#4000)	1.69	5.75	2.10
3	1.0 (#4000)	1.98	5.91	3.76
4	1.5 (#4000)	2.16	8.36	4.15
5	0.5 (#2000)	1.28	2.95	1.89
6	1.0 (#2000)	1.39	3.49	2.17
7	1.5 (#2000)	2.03	5.10	3.16

Figure 1 The diffusion curves of glucose through membranes 1–4.

measurements (not shown). In addition, it was found that the mass transfer coefficient of all the diffusion molecules increased with an increase of polyethylene glycol in the casting solution and the effect of PEG #4000 was greater than PEG #2000. In order to investigate these permeation characteristics, the structure of membranes was examined by SEM. As shown in *Figures 2–5*, membranes 1–4 had a typical asymmetric structure: a thin skin layer supported by a porous sublayer. Membranes 5–7 also had the same structure (not shown).

Membrane 1 without polyethylene glycol in the casting solution showed a dense skin layer almost without any pores (*Figure 2*). From *Figures 3(b)*, *4(b)* and *5(b)*, however, it was evident that the pores in the skin layer increased with increasing amount of polyethylene glycol in the casting solution. The effect of polyethylene glycol on forming pores in the skin layer was attributed to the fact that water is a good solvent for polyethylene glycol. Therefore, as soon as the casting solution was immersed into the coagulation bath, polyethylene glycol near the surface was dissolved in the coagulation bath. Nevertheless, most of the polyethylene glycols were still embedded in the interior when membrane was formed. As a result, the structure of the outermost top layer was relatively dense. If the incipient membrane continued to be kept in the coagulation bath for a longer period, water could diffuse through the outermost top layer. Consequently, polyethylene glycols were released with a dissolution-diffusion mechanism and they created pores which were originally occupied by polyethylene glycols in the incipient membrane. Therefore, the top-layer of membranes 3 and 4 could be considered as consisting of two parts: the outermost dense layer and a layer full of pores. In other words, the 'true' skin layer became thinner. It was implied that the permeation characteristics were greatly affected by the amount of polyethylene glycol in the casting solution, due to the principal diffusion resistance in the 'true' skin layer.

Figure 2 SEM pictures of membrane 1. **a**, Top; **b**, cross-section.

Figure 3 SEM pictures of membrane 2. **a**, Top; **b**, cross-section.

Figure 4 SEM pictures of membrane 3. **a**, Top; **b**, cross-section.

Figure 5 SEM pictures of membrane 4. **a**, Top; **b**, cross-section.

Figure 6 The *in vitro* insulin secretory response of the islets after glucose stimulus separated by membrane 4.

Membrane 4 was the most effective in being permeated by glucose and insulin; therefore, the *in vitro* insulin secretory response of the islets after glucose stimulus separated by membrane 4 was studied and is shown in Figure 6. A slow increase of glucose concentration but a rapid increase of insulin concentration was observed in the islet-side. The insulin concentration in the glucose-side indicated a gradual increase to reach a plateau at approximately 30 min. This finding indicated that the islets prevented by membrane 4 could respond to a glucose stimulus and secrete insulin. As shown in Figure 7, the artificial islet system could be considered with the aid of control theory as a dynamic model which combined three systems in series:

System 1: diffusion of glucose from the glucose-side through the membrane to the islet-side.

System 2: secretion of insulin by islets.

System 3: diffusion of insulin from the islet-side through the membrane back to the glucose-side.

The islet system could be regarded as an independent unit. Several insulin-secretion models are available^{18–20}. From Figure 6, the glucose concentration rise of the islet-side was gradual, like a ramp input process for system 2. However, significant insulin-secretion occurred, and then gradually moderated. This indicated that brief exposure of the islets to glucose induces a rapid release of insulin and with continuous glucose

Figure 7 Block diagram of the artificial islet model.

exposure, the initial response fades, corresponding to the second phase insulin secretion²¹.

Using the Laplace transformation, Equation (1) can be expressed as

$$\frac{C_1(S)}{C_1(S)} = \frac{1}{1 + TS} \quad (3)$$

T indicating the time constant ($T = LV/AD$). For systems 1 and 3, the time constant was calculated to be about 420 min and 22 000 min for diffusion of glucose and insulin through membrane 4, respectively. Both of these values are much greater than the time constant of the islets, 12 min, proposed by Nomura *et al.*²⁰. Since diffusion and insulin-secretion processes were involved in the overall process, the relative rate was important. Therefore, when the insulin-secretion rate was relatively high compared to the rate of diffusion, the process was said to be diffusion-controlled. In addition, diffusion of glucose was faster than that of insulin; thus, the process is insulin-diffusion-controlled. Hence, it would be possible to obtain a less dense skin layer to increase the mass transfer coefficient of insulin to reduce the time lag of the artificial islets; however, the less dense structure may lead to the passage of antibodies and immune cells. Therefore, it was important to investigate whether the structure of poly(vinyl alcohol) membranes, in particular the outermost dense layer, was totally impermeable to larger molecules such as IgG. In our studies, no permeation of IgG through the poly(vinyl alcohol) membranes 1–7 could be detected within the experimental time frame. Theoretically such membranes, thus, could completely avoid the permeation of antibodies and immune cells.

CONCLUSIONS

To ensure the rapid response of islets prevented by an artificial membrane, the membrane should have high permeability for glucose and insulin. Although low permeability of poly(vinyl alcohol) membrane to insulin was an obstacle to the artificial islets, we had proposed modifying the properties of the skin layer to improve insulin permeation. Adding polyethylene glycols to the poly(vinyl alcohol) membrane formation system, phase inversion process occurred first to determine the membrane structure with a dense skin layer supported by a porous sub-layer. Subsequently, polyethylene glycols were released to create pores in the skin layer. Since the 'true' skin layer became thinner by increasing the amount or molecular weight of polyethylene glycol in the casting solution, it was possible to obtain poly(vinyl alcohol) membranes with quite precisely identified permeation properties. This study clearly showed the effect of the structure of the skin layer on the permeation characteristics of the membranes. Furthermore, the size difference among the penetrant molecules in the artificial islets is quite large, so the 'true' skin layer could still function adequately as an immunoprotective barrier. These preliminary data were consistent with the potential interest for the development of artificial islets.

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