# 行政院國家科學委員會專題研究計畫成果報告

## 聚乙烯醇薄膜應用於人工胰臟的動物實驗

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**一、中文摘要**

因聚乙烯醇具有生物不活潑性質，且由之前的研究證實了聚乙烯醇薄膜適用於人工胰臟。本計畫進一步將聚乙烯醇薄膜應用於人工胰臟的動物實驗，可觀察出在植入 M-2 管子之後，糖尿病老鼠的血糖濃度從 500±35 降到 210±22 mg/dl，經由植入三組 M-2 管子之後，更可以降到 130-160 mg/dl 且可維持一個月。最後利用數學動力模型來解釋之。

**關鍵詞：人工胰臟，糖尿病**

**Abstract**

Our previous in vitro studies have demonstrated that poly (vinyl alcohol) (PVA) membranes satisfy the basic requirements for a bioartificial pancreas. This experiment was designed to evaluate the performance of m-1 and m-2 tubular membrane chambers containing islets in vivo. When the m-2 chamber was implanted into streptozotocin (STZ)-induced diabetic rats, nonfasting blood glucose levels dropped from 500±35 to the lowest value 210±22 mg/dl. Furthermore, the performance of the bioartificial pancreas can be enhanced by the increased numbers of implanted chambers. If three m-2 chambers was implanted, nonfasting blood glucose levels in the diabetic rats decreased to 130-160 mg/dl and such a low blood glucose value was maintained for 1 month. In addition, a mathematical kinetic model was developed to describe the characteristics of islet inside an artificial environment.
**Keywords**: bioartificial pancreas, diabetes.

**INTRODUCTION**

Long term blood glucose normalization in diabetes mellitus is an important goal. Much effort has focused on the development of the bioartificial pancreas, in which the transplanted pancreatic islet tissue is enclosed within a semipermeable membrane. Our previous in vitro studies have demonstrated that poly (vinyl alcohol) (PVA) membranes satisfy the requirements for a bioartificial pancreas: rapidly releasing insulin through the membranes in response to changes in concentrations of glucose in the dynamic perifusion experiment but the passage of immunoglobulin G was completely prevented.[1,2] In this work, data obtained from in vivo transplantation studies supported the previous assessment and the performance of the bioartificial pancreas can be enhanced by the increased numbers of implantated chambers.

**MATERIALS AND METHODS**

M-1 and m-2 PVA (molecular weight 74,800) tubular membrane chambers were constructed according to the procedure described previously.[1,2] Each PVA chamber seeded with about 80 islets was implanted into the peritoneal cavity of three streptozotocin (STZ)-induced diabetic rats. Nonfasting blood glucose concentrations were measured by tail bleeding using a glucose analyzer.

**Mathematical model**

For improving the design of the bioartificial pancreas, a mathematical model was developed to account for the changes in blood glucose levels of the diabetic rats. The theoretical analysis describing the initially rapid decrease of blood glucose concentration, maintaining a constant level and the finally rapid rise of blood glucose concentration was derived as follows. Let N be the number of islets placed in a chamber, and C be the blood glucose concentration of a rat. Initially, 80 islets were seeded in each chamber. In the

previous perifusion study,[2] the contribution of most islets in the chamber was insignificant. Due to the fact that only some of the islets functioning to secrete insulin, we assume that there exists a critical number of islets, $N_c$. If $N > N_c$, the number of effective (working) islets becomes $N_c$. In this case, the rate of decrease in the blood glucose concentration is proportional to $N_c$. Also, suppose that the blood glucose concentration has a lower bound, $C_k$, which can be estimated on the basis of experimental data. That is, the blood glucose concentration can not be lower than a minimum level. On the other hand, if $N < N_c$, the number of effective islets is N, and the blood glucose concentration will increase with time, and we assume that the rate of increase is proportional to $(N_c-N)$. Concerning the decrease in the number of islets, a reasonable result is most likely preceded by a progressive decrease in B-cell mass. Either of the aggregation of the islets inside the chamber or the PVA chamber encapsulated by connective tissue could contribute to increased necrotic islet cells to result in the number of islets decreasing with time. Here, we assume that the rate of decrease in the number of islets is proportional to N. Based upon the above discussions, the temporal variations in the blood glucose concentration and the number of islets can be described by

$$-\frac{dC}{dt} = k_1 N_c \text{ , } N>N_c \qquad (1)$$

$$C = C_k \text{ , } C<C_k \qquad (2)$$

$$\frac{dC}{dt} = k_2 (N_c - N) \text{ , } N<N_c \qquad (3)$$

$$\frac{dN}{dt} = -k_3 N \qquad (4)$$

Here, t denotes time and $k_1$, $k_2$, and $k_3$ are rate constants, which are related to the characteristics of an artificial pancreas.

## RESULTS AND DISCUSSION

In the diabetic rats without any peritoneal transplantation, nonfasting blood glucose levels showed a hyperglycemic state (about 460-500 mg/dl) and no significant changes; see Figure 1. In the three diabetic rats receiving m-1 chamber, nonfasting blood glucose levels dropped from 500 ± 35 to the lowest value 340 ± 20 mg/dl. It could not reverse the hyperglycemic state and it only maintained insulin secretion activity for 10 days. In contrast, nonfasting blood glucose levels in recipient diabetic rats were significantly decreased after the intraperitoneal transplantation of the m-2 chamber. The lowest value obtained was about 210 ± 22 mg/dl. Rats sustained nonfasting blood glucose levels less than 300 mg/dl for about 20 days. This indicates that the m-2 chamber could provide improved permeability of insulin to normalize blood glucose levels and improved protection of islets from the immune system of the recipient. This result is consistent with the effect of adding polyethylene glycol to the m-2 chamber to create pores in the skin layer, leading to increased membrane permeability. Furthermore, the number of implanted m-2 chambers was increased to improve the control of blood glucose levels. If three m-2 chambers were implanted in the diabetic rats, nonfasting blood glucose levels in the diabetic rats decreased to 130-160 mg/dl and such a low blood glucose value was maintained for 1 month (Figure 2). Thus, the survival of islets was better for more-chamber than one-chamber implantation. In summary, implanting three m-2 chambers in the diabetic rats is good enough for glycemic normalization.

In the simulation, the best fitted parameters, $k_1$, $k_2$, $k_3$ and $N_c$ were estimated and summarized in Table 1. Regardless of the number of the m-2 chambers implanted in the rats, the $k_1$, $k_2$ and $k_3$ values do not have significant difference, however, these three parameters have significant difference

between the m-1 and m-2 chambers. The rate constant $k_1$ in equation (1) mainly depends upon the permeation rate of insulin secreted by islets through the membrane, which is determined by the skin layer structure of the membrane.[1,2] The larger the value of $k_1$ the better the performance of a device. As can be seen in Table 1, the performance of the m-2 chamber is better than that of the m-1 chamber. This result is consistent with the effect of adding polyethylene glycol to the m-2 chamber to create pores in the skin layer, leading to increased membrane permeability. In addition, the definition of $N_c$, given in equation (1), suggests that the larger its value the more insulin secreted by effective islets, and the better the performance of a device. According to Table 1, the performance of the devices examined, from the best to the worst, follows the order m-2(III) > m-2(II) > m-2(I) > m-1. (The letters in parentheses represent the number of implanted m-2 chambers.) Combining the effect of $k_1$ with $N_c$, the performance of the devices examined, from the best to the worst, follows the order m-2(III) > m-2(II) > m-2(I) > m-1.

The definition of $C_k$, estimated by a linear regression analysis on the experimental blood glucose concentrations, implies that the lower its level the better the performance of a device. The level of $C_k$ for each device follows the order m-2(III) < m-2(II) < m-2(I) < m-1, as indicated in Table 1. That is, the performance of the devices examined, from the best to the worst, follows the order m-2(III) > m-2(II) > m-2(I) > m-1, which is consistent with the combined effect of $k_1$ and $N_c$. Theoretically, the greater the value of $k_1$ multiplied by $N_c$, the lower value of $C_k$. Figure 4 shows the level of $C_k$ seems to have a limiting value about 170 mg/dl as the value of $k_1$ multiplied by $N_c$ (or the number of implanted m-2 chambers) increases. Therefore, implanting three m-2 chambers in the diabetic rats is good enough for glycemic normalization.

Equation (3) suggests that the larger the value $k_2$, the faster the rate of increase in blood glucose concentration, and the worse the performance of a device. According to Table 1, the $k_2$ value of the m-1 chamber is greater than that of the m-2 chamber. It is reasonable that $k_1$and $k_2$ have the opposite tendency from the view point of the membrane permeability.

Equation (4) shows that $k_3$ is related to the rate of decrease of the number of islets; the smaller its value the lower the rate of decease of the number of islets, and the better the performance of a device. Table 1 implies that the $k_3$ value of the m-2 chamber is less than that of the m-1 chamber. Another parameter describing the effect of the $k_3$ is the time interval, $T_o$, in which the blood glucose concentration can be maintained at a certain low level. When the number of "viable" islets was less than $N_c$, the level of blood glucose concentration is rapidly elevated. Apparently, the larger the $T_o$ the better the performance of a device. Table 1 reveals that the performance of the devices examined, from the best to the worst follows the order m-2(III) > m-2(II) > m-2(I). Thus, the survival of islets was better for multiple-chamber implantation than single-chamber implantation. Prolonged survival of islets is probably related to the diminished excessive demands on its function from persisting stimulus of high blood glucose levels. In addition, Table 1 shows each chamber had smaller value for $N_c$ when the number of implanted chambers increased. This also suggests the multiple-chamber implantation can satisfy the requirement of $N > N_c$ for a longer period.

In summary, we conclude that the performance of m-2(III) is better than that of the other three types of devices.

## REFERENCES


1. TH Young et al., Biomaterials, 17, 2131 (1996.)
2. TH Young et al., J. Biomed. Mater. Res., 40, 385 (1998.)


TABLE 1. Summary of the estimated

parameters in fitting the experimental data shown in Figure.3.

| | m-1 | m-2 (I) | m-2 (II) | m-2 (III) |
|---|---|---|---|---|
| $k_1$ | 4.2 | 4.5 | 4.7 | 4.7 |
| $k_2$ | 1.6 | 0.56 | 0.54 | 0.53 |
| $k_3$ | 0.22 | 0.067 | 0.065 | 0.065 |
| $N_c$ | 22 | 24 | 28 | 30 |
| $T_o$ | 4.5 | 16 | 24 | 30 |
| $C_k$ | 369 | 256 | 183 | 173 |

Figure 1. Nonfasting blood glucose levels of diabetic rats, diabetic rats transplanted with 200 free islets, and diabetic rats transplanted with 80 islets seeded in the m-1 and m-2 tubular membrane chamber.

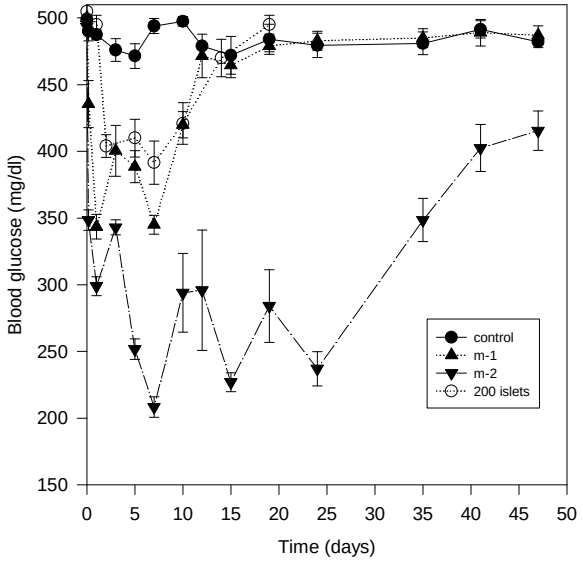


Figure 2. Comparative study on changes in nonfasting blood glucose levels among diabetic rats transplanted with one, two and three m-2 tubular membrane chambers.