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添付資料 研究報告書


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２．研究テーマ

膵島の長期保存及び皮下移植における polyvinyl alcohol (PVA)マクロカプセル化膵島の応用

３．成果の概要

膵島の長期保存におけるＰＶＡマクロカプセル化膵島の in vitro 及び in vivo にての有用性を確認し、報告した。膵島の皮下移植における PVA マクロカプセル化膵島の応用の実験の一部はなお進行中であるが、近日中に終了する予定である。

４．研究業績

（１）学会における発表　　有（学会名・演題）

学会名：　The 40th Anniversary Meeting of American Pancreatic Association and Japan Pancreas Society

演題名　Cryopreservation of polyvinyl alcohol (PVA)- encapsulated islet

（２）発表した論文　　有（雑誌名・題名）

雑誌名　Biomaterials

題名　The in vivo performance of polyvinyl alcohol macro-encapsulated islets

— 日中医学協会助成事業—

# 膵島の長期保存における polyvinyl alcohol (PVA)マクロカプセル化膵島の応用

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**要旨**

Islet transplantation is a method for the treatment of type 1 diabetes mellitus (DM) and has been widely performed around the world. The long-term cryopreservation of islets shows many advantages in the field of islet transplantation. Previous studies have described the development of sheet-type polyvinyl alcohol (PVA) macro-encapsulated islet (MEI) to treat type 1 DM without any immunotherapy. The present study examined their beneficial effects on islet cryopreservation. PVA MEI of Wistar rats were divided into three groups of 1-day, 7-day and 30-day cryopreservation at -80℃. The 30-day group showed a lower recovery rate of the islet number and impaired insulin release in comparison to the 1-day group, whereas no significant differences of the *in vitro* results were observed between the 1-day and 7-day groups. The MEI transplantation recipient mice in the 1-day and 7-day groups reached normoglycemia for a 4-week observation period, and the recipients in 30-day group also showed a significant decrease followed by a slightly higher non-fasting blood glucose level. These results suggest that the PVA MEI are useful for islet long-term cryopreservation, and that the use of cryopreserved PVA MEI may therefore be a promising modality for performing DM therapy.

**Key Words** Polyvinyl alcohol (PVA), Macro-encapsulated islet (MEI), Islet cryopreservation, Islet transplantation.

**緒言：**

Islet transplantation is a method for the treatment of type 1 diabetes mellitus (DM) and has been widely performed around the world [1]. The long-term cryopreservation of islets shows many advantages in the field of islet transplantation: 1. Isolated islets can be shipped to other institutions worldwide. 2. Islets isolated at different time can be accumulated to obtain a sufficient number for transplantation.3. Cryopreservation provides the time for quality control of islets before transplantation [2]. This study tested 2 periods (7 days and 30 days) of freezing and examined their functions *in vitro* and *in vivo* in comparison to the original method of cryopreservation (1-day freezing).

**対象と方法：**

PVA MEI of Wistar rats were divided into three groups of 1-day, 7-day and 30-day cryopreservation at -80℃. Morphological changes of islets, islet recovery rate and insulin secretion test were performed in vitro. In vivo, Eight hundred encapsulated Wistar rat islets were transplanted into the peritoneal cavity of diabetic C57BL/6 mice. Non-fasting blood glucose (NFBG), the body weights, and IPGTT were observed after transplantation. Blood samples were collected from the sacrificed mice 4 weeks after transplantation, serum insulin and c-peptide levels were measured. Transplanted MEI and recipients' pancreas were retrieved from mice sacrificed 4 weeks after transplantation, HE and insulin staining were performed.

**結果：**

1 .Morphological changes and islet recovery rate

The MEI in the three groups showed a normal morphology after freezing-thawing, without islet fragments, and no obvious differences were observed between the three groups. The islet recovery rate in the 1-, 7- and 30-day groups were 74.4±1.72%, 69.6±3.97% and 62.8±3.2%, respectively (7-day vs. 1-day: $p>0.05$; 30-day vs. 1-day: $p<0.05$).

2. Static incubation

The MEI in the three groups showed good insulin secretion abilities in response to high glucose concentration. The stimulation index in the 1-, 7- and 30-day groups was 1.84±0.07, 1.71±0.1 and 1.66 ±0.07, respectively. No significant differences were found between three groups. However, the insulin release in the basal (3.3 mM) and stimulation (16.7 mM) medium of the 30-day group was lower than 1-day group ($p<0.05$).

3. MEI xeno-transplantation

Mice in the 1-, 7- and 30-day groups showed a significant decrease in the NFBG levels in comparison with those in DM group after PVA MEI xeno-transplantation. Moreover, mice in the 1- and 7-day groups achieved normoglycemia (NFBG<200mg/dl) within 1 week after transplantation, and maintained normoglycemia for 4 weeks. Although mice in the 30-day group did not achieve normoglycemia, the NFBG significantly decreased from 485.8±25.1mg/ml to 246.3±19.6mg/dl (at the 4th week) after transplantation. The MEI groups maintained their body weight for 4 weeks. In contrast, the DM group showed a significant decrease in body weight in a time-dependent manner.

4. IPGTT

IPGTT was performed 2 weeks after transplantation. The 30-day group and DM group showed significantly higher area under the curve (AUC), and the normal group showed a significantly lower AUC in comparison to the 1-day group. No significant difference was observed in the AUC between the 1-day and 7-day groups. Moreover, the AUC in 30-day group was lower than that in DM group ($p<0.05$).

5. Serum insulin and C-peptide

The 1-, 7- and 30-day groups showed higher serum insulin and *C* -peptide concentrations than the DM group ($p<0.05$), and no significant differences were observed among the 1-, 7- and 30-day groups.

6. Histological findings

HE staining of the pancreas of recipient mice was performed in each group to check the regeneration of islets in STZ-induced diabetic mice. No intact islets were observed in the STZ-induced diabetic mice (DM, 1-, 7- and 30-day groups). In contrast, large islets with intact morphology were found in the normal group. These results indicated that the regeneration of islet did not happen in the STZ-induced diabetic mice. Insulin staining was performed in the MEI group (1-, 7- and 30-day groups) to confirm the surviving islets in the PVA MEI 4 weeks after transplantation. The islets in each MEI group were positive for insulin staining.

**考察**：

Although MEI in the 30-day group showed a slightly worse function *in vitro* in comparison to that seen in the 1-day group, and the recipient mice in the 30-day group did not achieve normoglycemia, there were still some therapeutic benefits with 30-day cryopreserved PVA MEI in comparison to the DM group *in vivo* . In fact, the survival rate of recipients 4 weeks after transplantation was 100% in the 30-day group and 17% in the DM group. The results of the NFBG, body weight and IPGTT in the 30-day group also showed apparent improvements from the DM group. In addition, the MEI in the 7-day group showed similar results to the 1-day group *in vitro* and *in vivo*. These results indicated that the immediate use of PVA MEI after 1 day freezing is not mandatory, furthermore, 7 days is sufficient for islet accumulation for transplantation, islet shipping worldwide and an evaluation of islet quality before transplantation. These results lead us to conclude the use of PVA MEI therefore appears to be an effective modality which can be used for clinical islet transplantation in the near future.

**結論**：

Long- term cryopreserved PVA MEI showed similar effects to the original PVA MEI (1-day group) both *in vitro* and *in vivo*. These results suggest that the PVA MEI have advantages over other MEI which may therefore make it possible to overcome the obstacles of insufficient donors and the side effects of immuno-suppressive drugs, because the encapsulation process with cryopreservation technique allows islet accumulation, as well as the shipping and quality control in the field of islet transplantation. Therefore, the use of PVA MEI appears to be an effective modality for improving clinical DM therapy.

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注： 本研究は、２００９年１１月６日 「The 40th Anniversary Meeting of American Pancreatic Association and Japan Pancreas Society」にてポスター発表、「Biomaterials」（In Press）に掲載。

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