MULTIPLE PEGYLATION OF PANCREATIC ISLETS FOR IMMUNOPROTECTION IN ISLET TRANSPLANTATION

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ABSTRACT

We studied the viability and function of islet with monomethoxy polyethylene glycol (mPEG) grafted onto its membrane. Islets were isolated from rat and were repeatedly reacted with activated mPEG (mw 5000) in order to increase grafting density. The density of grafted PEG on the islet membrane was confirmed by Fluorescein-PEG-NHS. An assessment of islet viability using AO / PI staining method showed that multiple PEGylation did not reduce islet viability. The function of PEG grafted islets was evaluated by measuring released insulin from islets. Insulin secreted from the PEGylated islets for 1 h did not show any significant difference compared to control (non-PEGylated) islets. In addition, PEGylated islets responded in the same pattern as control islets in the perifusion test.

INTRODUCTION

Immunoisolation devices proposed for islet transplantation can be classified into intravascular

device, macrocapsules, and microcapsules [1]. We have tried to enhance the viability and immunoprotection of transplanted islets. In this study, we modified the membrane of islets with biocompatible polymer such as polyethylene glycol (PEG) [2]. The presence of PEG at the surface of the islets [3], islet viability [4], and the function of islets [5, 6] after multiple PEGylation are investigated.

MATERIALS AND METHODS

Pancreatic islets were isolated from SD rat by the collagenase digestion method and cultured in RPMI-1640 medium for two days in order to recover the damaged membrane from isolation procedure. The PEGylation of islet was performed in HBSS solution under 5% CO_2 at 37°C. After PEGylation for 1 h, PEGylated islets were cultured for 1 day. The islets were PEGylated again for 1 h and cultured for another day. The PEGylation of islets was performed 3 times. To evaluate the density of PEG binding to islet membrane, islets were reacted with FITC-PEG and observed by a laser confocal scanning microscopy. The viability of multiple PEGylated islets was determined by a rapid fluorometric method using acridine orange and propidium iodide. Stained islets with AO / PI were classified according to their fluorescent colors. The function of PEGylated islets was investigated by monitoring insulin secretion from islets in static incubation. A perifusion assay was used to determine the ability of dynamic insulin secretion from multiple PEGylated islets in response to different glucose stimulation.

RESULTS

From the laser confocal scanning micrograph images, we observed that the more PEGylation we performed, the more density of grafted PEG increased. An assessment of islet viability using the staining method by AO / PI showed that multiple PEGylation did not reduce islet viability. Insulin release in static culture was used to evaluate multiple PEGylated islet function. Insulin secretion of PEGylated islets for 1 h did not show any significant difference compared to control islets (Fig.1). In addition, PEGylated islets responded in the same pattern as control islets in the perifusion test (Fig.2).

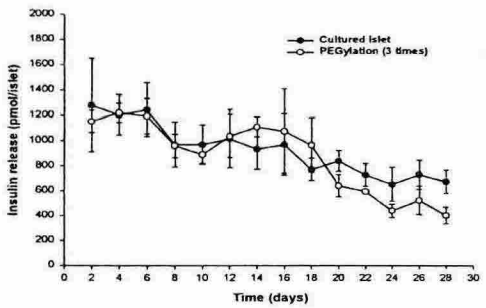

Fig. 1. Insulin release in static culture

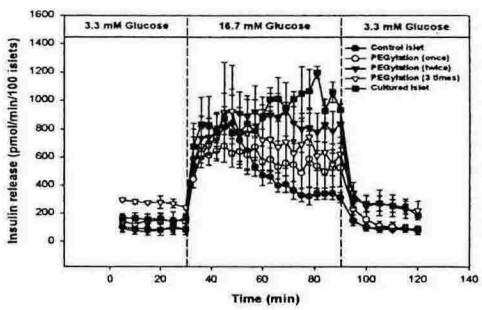

Fig. 2. Insulin release in perifusion test

CONCLUSIONS

Therefore, these results demonstrate that PEG polymer in islet transplantation can be used as a barrier for immunoprotection without any damage of islet function.

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