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Encapsulation of islets with ultra-thin polyion complex membrane through poly(ethylene glycol)-phospholipids anchored to cell membrane

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Abstract

The microencapsulation of islets of Langerhans (islets) has been studied as a safe and simple technique for islet transplantation without the need for immuno-suppressive therapy. However, thinner membranes are desired, because the increased total volume of the implant led to limited transplantation sites. Here, we propose a novel method for microencapsulation by polyion complex membrane formation on islets. Amino group-terminated poly(ethylene glycol)-conjugated phospholipids (PEG-lipids, M_w: 5000) spontaneously formed a thin layer on cells existing in the outer layer of islets when they were added to islet suspension. This layer-by-layer membrane could be further formed on the PEG-lipid layer through polyion complex formation between amino groups at the end of PEG chains, sodium alginate and poly(L-lysine). Islets could be microencapsulated by this method without volume increase. Encapsulation of the islet surface with PEG-lipids and polyion complex membranes did not impair the insulin release function in response to glucose stimulation. Our method is promising to encapsulate islets without affecting cell viability or increasing volume.

Keywords: Bioartificial pancreas; Islet; Poly(ethylene glycol)-lipid (PEG-lipid); Polyion complex; Microencapsulation

1. Introduction

The encapsulation of living cells with semi-permeable membranes has been studied as a safe and simple technique for cell transplantation without the need for immuno-suppressive therapy, especially in the transplantation of encapsulated islets, that is, bioartificial pancreas, for insulin-dependent diabetes mellitus. Several studies have been done on bioartificial pancreas, where islets were microencapsulated with an alginate-poly(L-lysine) polyion complex membrane [1–4] and agarose hydrogel to protect them from the host immune response [5–7]. However, some serious issues remain to be solved; the total volume of the implant was increased, which limited the selection of transplantation sites. For example, when microencapsulation increases the diameter of islets five-fold, the total volume increased 125-fold. In the clinical setting, 10 mL (containing about 5×10^5 islets) of islet suspension is transplanted; therefore, the total volume of the encapsulated islets is calculated to be more than 1 L, making it impossible to implant the required amounts of microencapsulated islets. Moreover, to transplant into portal veins, the diameter of encapsulated islets would need to be much smaller; therefore, methods for the microencapsulation of islets without increasing the diameter of the implant are required.

Poly(ethylene glycol)-phospholipid conjugates (PEG-lipids) have been widely used for the surface modification of phospholipid vesicles (liposomes) to improve biocompatibility and prolong the circulation time in vivo [8–12]. Recently, PEG-lipid derivatives were used for protein anchoring in a single cell [13–15]. These studies demonstrate that PEG-lipids are useful molecules to modify cell surface properties. In this study, we proposed a novel method to microencapsulate islets with ultra-thin polyion complex membranes through PEG-lipids anchored to the cell membrane.

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2. Materials and methods

2.1. Materials

α-*N*-hydroxysuccinimidyl-ω-*tert*-butoxycarbonyl poly(ethylene glycol) (NHS-PEG-Boc, M_w: 5000) was purchased from Nektar Therapeutics (San Carlos, CA). 1,2-dipalmitoyl-*sn*-glycerol-3-phosphatidylethanolamine (DPPE) was purchased from NOF Corporation (Tokyo, Japan). Trifluoroacetic acid (TFA) was purchased from Wako Pure Chemical (Osaka, Japan). Chloroform, triethylamine, diethyl ether, sodium alginate (M_w: 300 kDa), and poly(L-lysine) (0.1 wt%, M_w: 150–300 kDa) were purchased from Nacalai Tesque (Kyoto, Japan). Fluorescein isothiocyanate was purchased from Dojido Laboratories (Kumamoto, Japan). Hanks' balanced salt solution (HBSS), phosphate-buffered saline (PBS), minimum essential medium (MEM), and Medium 199 were purchased from Invitrogen Co. (Carlsbad, CA). PD-10 columns were purchased from Amersham Biosciences (Uppsala, Sweden). Fetal bovine serum was purchased from BioWest (Miami, FL). Insulin assay was performed by an enzyme-linked immunosorbent assay (ELISA) (Shibayagi Co. Ltd., Gunma, Japan).

2.2. Synthesis of NH_2-PEG-lipid

PEG-lipid was synthesized from NHS-PEG-Boc and DPPE. An *N*-hydroxysuccinimidyl group is an ester activated to react with an amino group of DPPE, and Boc is a protective group for an amino group. NHS-PEG-Boc (185 mg), DPPE (21 mg), and triethylamine (3 μL) were dissolved into 10 mL of dichloromethane solution, and stirred for 24 h at room temperature. Then, a solution of TFA (2 mL) was added to the resultant solution for 30 min at 4 °C to remove Boc groups. The crude product was purified by reprecipitation in diethyl ether. After extraction into chloroform and evaporation, the PEG-lipid was obtained as a white solid (114.5 mg, yield 74%). For fluorescent labeling of PEG-lipids, FITC (3.1 mg) was reacted with PEG-lipids (20 mg) in acetone for 12 h. FITC-PEG-lipid was purified by gel permeation chromatography (Sephadex G-25). TLC (silica gel, chloroform/methanol) 4/1, v/v): R_f 0.6. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ ppm): 0.85 (*t*, 6H, $-CH_3$), 1.23 (br, 56H, $-CH_2-$) 3.65 (br, 460 H, PEG).

2.3. Incorporation of PEG-lipids into the surface of living cells

Human embryonic kidney cell line (HEK293) was used. After the dispersion of FITC-PEG-lipids in HBSS was added to HEK293 cell suspension (5×10^5 cells, [PEG-lipids] = 1, 10, 100, 1000 μM), the suspension was incubated for 120 min at 37 °C. This cell suspension was then washed with PBS and centrifuged (180*g*, 5 min, 25 °C, twice) to obtain FITC-PEG-modified cells. These cells were observed by a confocal fluorescence microscope (FLUOVIEW FV500, Olympus, Tokyo). A series of cross-sections was placed in a horizontal direction by 1.5 μm to obtain horizontal sectional images of FITC-PEG-modified cells using image-processing software (FLUOVIEW, Olympus). As a control experiment, a solution of FITC in HBSS (10 μM) was added to HEK 293 cell suspension (5×10^5 cells) at 37 °C. Before using FITC, isothiocyanate groups of FITC were deactivated by incubation in HBSS for 24 h since FITC could react with membrane proteins on cells. After washing with PBS and centrifugation (twice), they were also observed using a confocal microscope.

Cell viability was assessed by the trypan blue exclusion method. PEG-modified cells were prepared by adding a solution of NH_2-PEG-lipids in HBSS (10, 100, 1000 μM) to the cell suspension (5×10^5 cells) for 120 min at 37 °C. Also, a solution of NH_2-PEG-lipids in HBSS (10 μM) was added to the cell suspension for 30, 60, and 120 min at 37 °C. PEG-lipid-modified cells were mixed with a solution of trypan blue and examined under a microscope.

2.4. The number of PEG-lipids incorporated into the cell surface

The fluorescence intensity of FITC-PEG-lipid dispersion (10, 20, 50, 100 nM) was measured using a fluorophotometer (F-2500, Hitachi, Co., Tokyo), and used as a standard sample. FITC molecules at the end of PEG chains were excited at 480 nm. Fluorescence intensity at 520 nm was collected to obtain the standard curve. The dispersion of FITC-PEG-lipids at 10 or 100 μM was added to HEK293 cell suspension at 37 °C for 120 min. FITC-PEG-modified cells were washed with PBS and centrifuged (180*g*, 5 min), and the fluorescence intensity of the FITC-PEG-modified cell suspension (5×10^5 cells/mL) was measured. The number of PEG-lipids incorporated into the cell surface was calculated from the standard curve and the fluorescence intensity of the FITC-PEG-modified cell suspension.

2.5. Stability of PEG-lipid anchoring to the cell membrane

The dispersion of PEG-lipids (100 μM) was added to an HEK293 cell suspension (5×10^5 cells) and incubated for 30, 60, and 120 min at 37 °C. Then, PEG-modified cells were cultured in MEM containing 10% FBS on a culture dish after washing. FITC-PEG-modified cells were also cultured on a glass bottom dish, and the adherent cells were observed by confocal microscopy and total internal reflection fluorescence (TIRF) microscopy (Olympus).

2.6. Encapsulation of PEG-modified islets

Islets were obtained from the pancreas of Syrian hamsters (8 weeks, female) by the collagenase digestion method. After the islets were maintained in culture medium (Medium 199 containing 10% FBS, 8.8 mM HEPES buffer, and 8.8 U/mL Heparin) for 6 days, a PEG-lipid dispersion was added to the islet suspension in HBSS (1.0×10^2 islets, [PEG-lipid] = 100 μM in HBSS) and the mixture was incubated at 37 °C for 1 h. After washing with HBSS and centrifugation (180*g*, 1 min), PEG-lipid-modified islets were obtained. A sodium alginate solution (0.1% in HBSS) was added to the PEG-lipid-modified islet suspension for 15 min at r.t. to induce an ion complex formation between NH_2-PEG and alginate. Cells were washed again with HBSS and centrifuged. Then, poly(L-lysine) solution (0.01% in HBSS, 3 min) and sodium alginate solution (0.1% in HBSS, 15 min) were used alternatively to cover islets with the corresponding polyion. For visualization under a confocal fluorescence microscope, FITC-PEG-lipid and FITC-labeled poly(L-lysine) were used for PEG-lipid-modified islets and encapsulated islets, respectively.

2.7. Glucose stimulation insulin release

Static glucose-responsive insulin assay (16) was done for PEG-lipid modified islets and encapsulated islets to assess the function of these islets, which were incubated 0.1 g/dL, in 0.3 g/dL and then 0.1 g/dL glucose in Krebs-Ringer solution for 1 h each at 37 °C, respectively. Insulin concentrations in the supernatant at each step were determined by ELISA.

3. Results and discussion

3.1. PEG-lipid anchoring to the cell membrane

First, we studied the surface modification of living HEK293 cells with PEG-lipids, and then examined the encapsulation of islets, which are cell aggregates (~150 μm), using PEG-lipids and polyion complex membranes.

A phospholipid, DPPE was reacted with hetero-bifunctional PEG having an activated ester group and a Boc group at each end to synthesize PEG-lipids as shown in