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Islet-encapsulation in ultra-thin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly(ethylene glycol)–lipids in the cell membrane

Yuji Teramura[a,b], Yoshihiro Kaneda[c], Hiroo Iwata[c,*]

[a]Department of Nano-Medicine Merger Education Unit, Graduate School of Engineering, Kyoto University, Nishikyo-ku, Kyoto 615-8510, Japan
[b]Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Nishikyo-ku, Kyoto 615-8510, Japan
[c]Institute for Frontier Medical Sciences, Kyoto University, 53 Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

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Abstract

The microencapsulation of islets of Langerhans (islets) in a semipermeable membrane, i.e., the creation of a bioartificial pancreas, has been studied as a safe and simple technique for islet transplantation without the need for immunosuppressive therapy. The total volume of the implant tends to increase after enclosure of the islets in the semipermeable membrane, which limits transplantation sites. Thus, ultra-thin membranes are required for clinical applications. Here, we propose a novel method to encapsulate islets in an ultra-thin membrane of poly(vinyl alcohol) (PVA) anchored to a poly(ethylene glycol) (PEG)–phospholipid conjugate bearing a maleimide group (Mal–PEG–lipids, PEG Mw: 5000) in the cell membranes of islets. When Mal–PEG–lipids were added to an islet suspension, they spontaneously formed a thin layer on cells of the outer layer of islets. The PEG–lipid layer on the islets was covered by a PVA monolayer, and the PVA membrane was further reinforced by using the layer-by-layer method with thiol/disulfide exchange reactions. No practical volume increase in islets was observed after microencapsulation by this method. In addition, encapsulation of the islet surface in PVA membranes did not impair insulin release in response to glucose stimulation.

Keywords: Bioartificial pancreas; Islets; Poly(ethylene glycol)–lipid (PEG–lipid); Poly(vinyl alcohol) (PVA); Layer-by-layer membrane

1. Introduction

Transplantation of islets of Langerhans (islets) enclosed in a semipermeable membrane (i.e., a bioartificial pancreas) has been proposed as a safe and effective method for treating insulin-dependent diabetes mellitus (type I) without the need for immunosuppressive therapy. Because the islets are isolated from the host immune system by a semipermeable membrane, they can survive and thus control glucose metabolism for a long period of time. Various types of bioartificial pancreas have been proposed and developed, including islets microencapsulated within an alginate/poly(L-lysine) polyion complex membrane [1–5] or agarose hydrogel [6–8] or macroencapsulated by porous membranes [9–11].

Recently, microencapsulated types of bioartificial pancreas have been preferentially studied due to efficient oxygen and nutrient supplies to cells in the islets [11–13]. However, some serious issues must be resolved, such as the increase in total volume of the implant upon encapsulation, which limits transplantation sites. When the diameter of capsules of islets increases 5-fold in our previous work [6], the total volume increased more than 100-fold. Based on this estimation, the total volume of the encapsulated islets is more than 1 L in the clinical setting (about 10 mL of islet suspension containing about 5×10^5 islets is now transplanted), making it impossible to implant the required amounts of microencapsulated islets. Much effort has been done to reduce the size of capsules. Calafiore et al. [14] reported much smaller microcapsules i.e. 300 mm diameter, which would make a total volume manageable for clinical application. The capsules with larger diameter than islet

*Corresponding author. Tel./fax: +81 75 751 4119.
E-mail address: iwata@frontier.kyoto-u.ac.jp (H. Iwata).

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itself, however, are expected to plug larger blood vessels than islets do. It imposes harmful effect on the patient liver. The diameter of encapsulated islets must be much smaller than that currently attained to allow transplantation of the islets into portal veins. Thus, new methods for the microencapsulation of islets without increasing the diameter of the implant are required.

Recently, poly(ethylene glycol) (PEG) chains covalently bound to membrane proteins of islets were investigated for immunoprotection of transplanted islets [15,16]. In another study, a polyelectrolyte complex membrane was formed on the negatively charged surface of islets by the layer-by-layer method [17] for the enclosure of islets in a very thin membrane. However, chemical modification by covalent bonding of PEG chains might damage membrane proteins, and most polycations are known to be cytotoxic. Thus, these two procedures are expected to negatively impact islet function.

PEG–conjugated phospholipids (PEG–lipids) have been widely used for the surface modification of phospholipid vesicles or liposomes to improve biocompatibility and to prolong the in vivo retention time [18–21]. Recently, PEG–lipid derivatives were used for anchoring proteins to a single cell [21–24] and for surface modification of islets [6]. The PEG–lipid derivatives are immobilized on the cell surface through the hydrophobic interaction between the lipid bilayer and the PEG–lipid. Cell surface modification with PEG–lipids is much more compatible with living cells than the two surface modification methods mentioned previously. However, we reasoned that a PEG layer would not be thick enough to cover cell surface antigens, and its mechanical properties would not be strong enough for long-term immunoprotection. Therefore, this study proposes a novel method to microencapsulate islets in an ultrathin membrane composed of poly(vinyl alcohol) (PVA) bearing thiol groups anchored to maleimide–PEG–conjugated lipids in the islet cell membranes.

2. Materials and methods

2.1. Materials

α-*N*-hydroxysuccinimidyl-ω-maleimidyl PEG (NHS–PEG–Mal, Mw: 5000) was purchased from Nektar Therapeutics (San Carlos, CA). 1, 2-Dipalmitoyl-*sn*-glycerol-3-phosphatidylethanolamine (DPPE) was purchased from NOF Corporation (Tokyo, Japan). 3,3′-Dithiodipropionic acid and dithiothreitol (DTT) were purchased from Wako Pure Chemical (Osaka, Japan). PVA ($n = 1700$) was kindly donated by Unitika, Ltd. (Osaka, Japan). Ethanol, hydrazine monohydrate, sodium nitrate, dimethyl sulfoxide (DMSO), chloroform, diethyl ether, sulfuric acid, heparin sodium salt, and 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent) were purchased from Nacalai Tesque (Kyoto, Japan). 2,2′-Dipyridyl disulfide was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Fluorescein isothiocyanate (FITC) was purchased from Dojindo Laboratories (Kumamoto, Japan). Minimum essential medium (MEM), HEPES buffer solution, RPMI1640 medium, and Medium 199 were purchased from Invitrogen Co. (Carlsbad, CA). Fetal bovine serum was purchased from BioWest (Miami, FL). Phosphate-buffered saline (PBS) was purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan).

2.2. Synthesis of thiolated PVA (PVA–SH)

PVA–SH was obtained by the reduction of PVA cross-linked by bis(β-isocyanatoethyl) disulfide (BIED) [25], a cross-linker having two isocyanato groups and an intramolecular disulfide bond. BIED was synthesized as summarized in Scheme 1a. 3,3′-Dithiodipropionic acid (10 g, 47.6 mmol) and ethanol (22.1 g, 480 mmol) were dissolved in toluene containing sulfuric acid (0.6%) and stirred for 15 h at 150 °C (flux) using a Dean-Stark trap to produce dithiodipropionate diethyl (**1**, yield 94%). Dithiodipropionate diethyl (1.0 g, 3.75 mmol) and hydrazine (1.2 g, 24.0 mmol) were dissolved in methanol (1.3 mL) and stirred for 1 h at r.t. to obtain dithiodipropionate dihydrazide after recrystallization from methanol (**2**, yield 74%). Dithiodipropionate dihydrazide (1.3 g, 5.4 mmol) was dissolved in HCl solution (5 mL water and 1 mL HCl) and stirred in an ice bath. After the addition of sodium nitrate solution (0.76 g, 11 mmol in 1 mL water) and toluene, the toluene solution was separated and incubated at 60 °C for 30 min. After the evaporation of toluene, BIED was obtained as a pale yellow solution (**3**, yield 53%). *^{1}H-NMR ($CDCl_3$; **1** and **3**, $(CD_3)_2SO$; **2**, 400 MHz, δ ppm): compound **1**: 1.33 (t, 3 H, -CH_3), 2.79 (t, 2 H, -OCO-CH_2-), 2.99 (t, 2 H, -S-CH_2-), 4.22 (q, 2 H, -O-CH_2-), compound **2**: 2.40 (t, 2 H, -NH-CH_2-), 2.89 (t, 2 H, -S-CH_2-), 4.21 (s, 2 H, -NH_2), 9.07 (s, H, -NH-), compound **3**: 2.86 (t, 2 H, -S-CH_2-), 3.61 (t, 2 H, NCO-CH_2-).*

BIED (2.7 mg) was added to a PVA solution (3 mL, 0.033 g/mL in DMSO) at r.t., and the solution gradually changed to a gel. After washing with pure water and DMSO, the gel was resuspended in DMSO. Then, DTT (10 mg) was added, and the suspension was stirred at 80 °C for 15 min to reduce the disulfide bonds. The gel gradually changed to a solution. The solution was added to acetone/HCl (pH 2), and the resulting precipitate was re-dissolved in DMSO and dialyzed against PBS to remove the DTT. PVA–SH was obtained after the dialysis (62 mg, yield 62%). The concentration of pendant thiol groups in PVA–SH was determined by Ellman's reagent. PVA–SH was stored under a nitrogen atmosphere at 4 °C until use. For visualization by confocal laser microscopy, PVA was labeled with FITC.

2.3. Synthesis of maleimide–PEG–conjugated DPPE (Mal–PEG–lipid)

Mal–PEG–lipid was synthesized from NHS–PEG–Mal and DPPE. NHS–PEG–Mal (180 mg) and DPPE (20 mg) were dissolved in chloroform and stirred for 24 h at r.t. After precipitation with diethyl ether, Mal–PEG–lipid was obtained as a white powder (190 mg, yield 80%). *^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm): 0.88 (t, 6 H, -CH_3), 1.25 (br, 56 H, -CH_2-) 3.64 (br, 480 H, PEG), 6.71 (s, 2 H, -HC = CH-, maleimide).*

2.4. Surface modification of single cells with PVA–PEG–lipid

An FITC-labeled PVA–PEG–lipid conjugate was used to modify the surface of CCRF-CEM cells (acute lymphoblastic leukemia T-cells). After reduction of the PVA gel by DTT, PVA–SH (30 mg) was reacted with Mal–PEG–lipid (20 mg) in PBS to prepare PVA–PEG–lipid conjugates. For visualization by confocal laser microscopy, PVA–PEG–lipid was labeled with FITC. After a solution of FITC-PVA–PEG–lipid conjugate (1.0 mg/mL in PBS) was added to a suspension of CCRF-CEM cells in RPMI1640 medium, the suspension was incubated at 37 °C for 10 min. Cells were washed with culture medium, collected by centrifugation, and observed by confocal laser microscopy.

2.5. Formation of PVA multilayers on islet cell surfaces

To introduce a pyridyl disulfide (PD) group to PVA–SH, 2,2′-dithiodipyridine (in methanol, 20 mg) was added to PVA–SH solution (3.0 mg/mL) and stirred at 4 °C for 12 h under a nitrogen atmosphere. The resulting PVA–PD was dialyzed against PBS.