Biomaterials 30 (2009) 2270–2275

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Islet encapsulation with living cells for improvement of biocompatibility

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

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

ARTICLE INFO

Article history:
Received 8 January 2009
Accepted 19 January 2009
Available online 6 February 2009

Keywords:
Bioartificial pancreas
Islet
Cell-encapsulation
Microencapsulation
Poly(ethylene glycol)–lipid (PEG–lipid)

ABSTRACT

Bioartificial pancreas, microencapsulation of islets of Langerhans (islets) within devices has been studied as a safe and simple technique for islet transplantation without the need for immuno-suppressive therapy. Various types of bioartificial pancreas have been proposed and developed such as microcapsule, macrocapsule and diffusion chamber types. However, these materials comprising a bioartificial pancreas are not completely inert and may induce foreign body and inflammatory reactions. The residual materials would be a problem in human body. Here we propose an alternative method for microencapsulation of islets with a layer of living cells. We immobilized HEK293 cells (human endoderm kidney cell line) to the islet surface using amphiphilic poly(ethylene glycol)-conjugated phospholid derivative and biotin/streptavidin reaction and encapsulated islets with a cell layer by culture. No necrosis of islet cells at the center was seen after microencapsulation with a layer of living cells. Insulin secretion ability by glucose stimulation was well maintained on these cell-encapsulated islets.

1. Introduction

Transplantation of islets of Langerhans (islets) has been proposed as a safe and effective method for treating patients with insulin-dependent diabetes mellitus (type I diabetes) although it was still an experimental procedure. In fact, the success achieved with the Edmonton protocol has established clinical islet transplantation as analternative to pancreas transplantation [1]. However, some improvements in islet transplantation are needed, such as increasing islet isolation efficiency, improving islets preservation, increasing the efficacy of immuno-suppressive drug dosage protocols, and reducing islet loss in the early phase following transplantation. A sufficient amount of islets for a recipient is isolated from a few donors. To overcome these issues, transplantation with islets enclosed in a semi-permeable membrane (i.e., a bioartificial pancreas) has been studied. Because islets are isolated from the host immune system by a semi-permeable 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 micro-encapsulated within an alginate/poly(L-lysine) polyion complex membrane [2] or agarose hydrogel [3–6] or macroencapsulated by porous membranes [7–9]. Our group has developed a microcapsule type of bioartificial pancreas using agarose hydrogel and demonstrated the efficacy in diabetic animals. Recently we also have originally developed a new bioartificial pancreas with fibrinolytic property for transplantation into the liver through portal vein [10–13].

However, the materials comprising a bioartificial pancreas are not completely inert and might induce foreign body and inflammatory reactions. The consecutive fibrous tissue overgrowth diminishes the diffusion properties of nutrients, waste products, and oxygen as well as hormones such as insulin. The residual materials would be a problem because islet transplantation must be repeated when the blood glucose level is not controllable by residual grafts. Therefore, when we consider the use of a bioartificial pancreas in the clinical setting, it is necessary to suggest a new concept.

Here we propose a new method for the microencapsulation of islets with a layer of living cells using amphiphilic polymers, which would create a novel bioartificial pancreas. It is expected that a cell layer formed on the islet surface would be an immunoisolation membrane. When using living cells derived from a recipient for islet encapsulation, it should be possible to improve the biocompatibility of islets after transplantation. In this study, a layer of living HEK293 cells was formed on the surface of islets using the biotin–PEG–lipid and biotin/streptavidin reaction shown in Scheme 1.

2. Materials and methods

2.1. Materials

α-*N*-Hydroxysuccinimidyl-ω-*tert*-butoxycarbonyl poly(ethylene glycol) (NHS–PEG–*Boc*, MW: 5000) was purchased from Nektar Therapeutics (San Carlos, CA).

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

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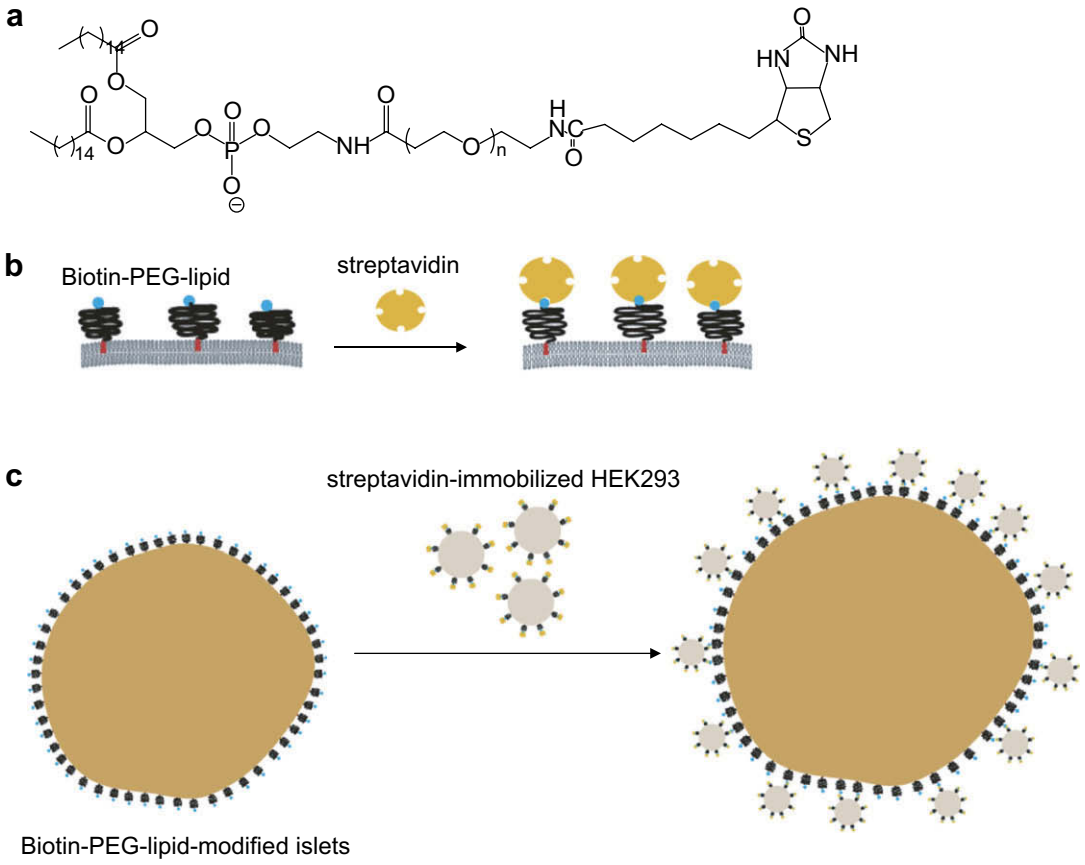
doi:10.1016/j.biomaterials.2009.01.036



Scheme 1. (a) Chemical structure of biotin–PEG-conjugated DPPE (biotin–PEG–lipid). (b) Schematic illustration of the interaction between streptavidin and biotin–PEG–lipid at the lipid bilayer cell membrane. Biotin–PEG–lipid has hydrophobic acyl chains and is incorporated into the cell surface by anchoring into the lipid bilayer. Streptavidin was immobilized on the cell surface by anchoring to biotin–PEG–lipid. (c) Scheme for the immobilization of streptavidin-immobilized HEK293 cells on the surface of biotin–PEG–lipid-modified islets. After mixing streptavidin-immobilized HEK293 cells and biotin–PEG–lipid-modified islets, they were cultured in medium at 37 °C on a culture dish. During culture, HEK293 cells were spread and grown on the cell surface to cover the whole surface.

1,2-Dipalmitoyl-*sn*-glycerol-3-phosphatidylethanolamine (DPPE) was purchased from NOF Corporation (Tokyo, Japan). Dichloromethane; chloroform; *N*,*N*′-dimethylformamide (DMF); diethyl ether; *N*,*N*′-dicyclohexylcarbodiimide (DCC); D-biotin; and streptavidin from *Streptomyces avidin* were purchased from Nacalai Tesque (Kyoto, Japan). Fluorescein isothiocyanate (FITC) and Hoechst 33342 were purchased from Dojindo Laboratories (Kumamoto, Japan). FITC-streptavidin was purchased from Zymed Laboratories (South San Francisco, CA). CellTracker®, the green fluorescent probe for the cell; Alexa 488-labeled goat anti-guinea pig IgG; minimum essential medium (MEM); HEPES buffer solution; Hanks' balanced salt solution; and Medium 199 were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from BioWest (Miami, FL). Phosphate-buffered saline (PBS) was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). The enzyme-linked immunosorbent assay (ELISA) kits for the insulin assay were purchased from Shibayagi Co., Ltd. (Gunma, Japan). Tissue-Tek was purchased from Sakura Fine Technical Co., Ltd. (Tokyo, Japan). Goat normal serum and polyclonal guinea pig anti-insulin were purchased from Dako (Glostrup, Denmark). The 10% formalin solution and Triton X-100 were purchased from Wako Pure Chemical (Osaka, Japan).

2.2. Synthesis of biotinylated PEG-conjugated DPPE

The NH_2–PEG–lipid was synthesized from DPPE and NHS–PEG–*Boc*, which carries an activated ester (NHS) that reacts with an amino group on DPPE, and a protective group (*Boc*) for the amino group, as reported previously [10]. Briefly, NHS–PEG–*Boc* (175 mg) and DPPE (21 mg) were dissolved in 5 mL dichloromethane solution and stirred for 3 days at room temperature (RT). Then, a solution of TFA (2 mL) was added and stirred for 20 min at 4 °C to remove the *Boc* groups. The crude product was purified by precipitation with diethyl ether. After chloroform extraction and evaporation, NH_2–PEG–lipid was obtained as a white solid (115 mg, yield 66%). D-Biotin (20 mg) and DCC (21 mg) were dissolved in DMF and stirred for 3 h at RT. Then, NH_2–PEG–lipid (22 mg) was added to the solution and stirred for 7 days at RT. This solution was filtered through a glass filter and DMF evaporated *in vacuo*. Chloroform was then added. After precipitation with diethyl ether, biotin–PEG–lipid was obtained as a white powder (10 mg, yield 40%). Biotin–PEG–lipid ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.88 (t, 6H, $-CH_3$), 1.25 (br, 52H, $-CH_2-$, DPPE), 3.18 (d, 2H, $C-CH_2-S$, biotin), 3.24 (q, 1H, S–CH(–C)–C, biotin), 3.64 (br, 460H, PEG), 4.52 (m, 2H, C–CH(–C)–N, biotin), 5.15 (s, 2H, C–NH–C, biotin).

2.3. Encapsulation of islets with HEK293 cells

HEK293 (human endoderm kidney cell line) cells were obtained from the Health Science Research Resources Bank (Tokyo, Japan). The HEK293 cells were maintained in MEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37 °C under 5% CO_2. The cells were collected by centrifugation (180 *g*, 5 min, RT) after treatment with trypsin. The cells were suspended in Hanks' balanced salt solution and then the medium exchanged. A cell pellet (4×10^6 cells) was obtained after centrifugation. After the addition of biotin–PEG–lipid solution (200 μL, 500 μg/mL) to the cell suspension, the suspension was incubated for 30 min with gentle agitation at RT. The cells were then suspended in 10 mL Hanks' balanced salt solution and collected by centrifugation (180 *g*, 5 min, 25 °C, twice) to obtain biotin–PEG–lipid-modified cells. Then, streptavidin (100 μL, 100 μg/mL) was added to the cell suspension and the suspension incubated for 30 min with gentle agitation at 4 °C. The cells were then suspended in 10 mL Hanks' balanced salt solution and collected by centrifugation (180 *g*, 5 min, 4 °C, twice) to obtain streptavidin-immobilized cells.

Islets were isolated from the pancreas of female Syrian hamsters (7–8 weeks old, Japan SLC, Inc., Shizuoka, Japan) using the collagenase digestion method. The islets were cultured for 7 days after isolation to remove or sediment cells damaged during the isolation procedure. The islets were maintained in culture medium (Medium 199 with 10% FBS, 8.8 mM HEPES buffer, 100 units/mL penicillin, 100 μg/mL streptomycin, and 8.8 U/mL heparin). A biotin–PEG–lipid solution was added to the islets suspended in serum-free MEM (200 islets, 500 μg/mL biotin–PEG–lipid, 100 μL of MEM), and the mixture was incubated at RT for 1.5 h. After washing three times with serum-free MEM, biotin–PEG–lipid-modified islets were obtained.

Finally, streptavidin-immobilized cells (4×10^6) and biotin–PEG–lipid-modified islets (200 islets) were mixed in serum-free MEM (300 μL), and the mixture was