

# Enhanced insulin secretion of physically crosslinked pancreatic $\beta$ -cells by using a poly(ethylene glycol) derivative with oleyl groups

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## Abstract

A polymeric crosslinker was developed to promote the formation of cellular spheroids. Our approach was based on the crosslinking of cell membrane using a polymeric crosslinker that worked via hydrophobic interaction. The crosslinker, a poly(ethylene glycol) derivative with oleyl groups as a hydrophobic group at both ends, was synthesized and characterized by gel permeation chromatography and Fourier-transform infrared spectroscopy. Cell culture experiments were then performed to confirm spheroid formation. The rat pancreatic islet  $\beta$ -cell line RIN, which possesses the ability to secrete insulin, was cultured with the crosslinker in a round-bottomed 96-well plate. The formation of a spheroid was achieved when the crosslinker was added to the cell suspension, especially in the absence of serum. The size of the spheroid decreased with time and with increasing crosslinker concentration, and depended on the number of cells plated in each well. The number of cells cultured with crosslinker was almost constant during 7 days and hardly proliferated in crosslinker concentrations of 0–2.5 mg ml<sup>-1</sup>, while the number of cells showed a decrease in the 25 mg ml<sup>-1</sup> crosslinker concentration. It was shown that the insulin protein secretion in the spheroid cultured with crosslinker for 1 week was enhanced. The cell adhesion protein E-cadherin mRNA expression of the resulting spheroid was also enhanced. These results indicate that the promoted cell function was due to the cell–cell and cell–matrix interactions in the spheroid, suggesting that this polymeric crosslinker was useful for the formation of cell spheroids.

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**Keywords:** Spheroid; Pancreatic cell; Poly(ethylene glycol); Aggregation; Crosslink

## 1. Introduction

Tissue engineering is an emerging technology that can be used to repair and regenerate damaged human tissue [1–3]. The technique of tissue engineering has taken a lead role in producing new tissue using materials and cells. There is a significant difference between a flat layer of cells and a three-dimensional tissue structure [4–7]. A three-dimensional cell culture is similar to conditions in vivo. The cell functions in a three-dimensional culture are increased because of the enhanced cell–cell interactions.

As the cells in a spheroid are known to possess enhanced functions compared with an individual cell, spheroids have great potential for applications in the field of tissue engineering. Recently, many approaches have been taken to form spheroids employing various techniques and materials, such as bioreactors [8–10], a spheroid-plate [11] and microfabricated surface-controlled cell adhesion [12]. However, they all required special devices and techniques, and the spheroids took a very long time to form. Therefore, the development of a convenient and speedy method for making spheroid is desirable. The objective of this study was to develop a novel polymeric crosslinker that can enhance the formation of spheroids.

A cell membrane is composed of a bilayer of amphiphilic phospholipids. Our approach was based on crosslinking a cell membrane using a polymeric crosslinker that works via

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hydrophobic interaction. The hydrophobic unit is a lipid, e.g., cholesterol and oleyl chain, that can anchor to the phospholipid bilayer of lipid membranes such as liposomes and cells [13–22]. Using hydrophobic interaction, the cell membrane can be anchored without causing any damage. The designed polymeric crosslinker consists of two distinct units: a hydrophobic unit, which can anchor to the phospholipid bilayer of a cell membrane, and a hydrophilic unit, comprising a polymer that promotes water solubility for use in aqueous media (Fig. 1a). In the present study, an oleyl group was the hydrophobic unit and a poly(ethylene glycol) (PEG) chain was the hydrophilic unit. We hypothesized that when this kind of polymeric crosslinker is added to cell suspensions, physical crosslinking would occur among cells via hydrophobic interaction (Fig. 1b).

In this work, the synthesis, characterization and evaluation of a novel crosslinker was performed to form cell spheroids. The rat pancreatic  $\beta$ -cell line RIN was used for the evaluation of spheroid formation. The small spherical cell masses called islets of Langerhans (pancreatic islets) are scattered throughout the pancreas and regulate the blood glucose level by secreting insulin. We first determined the effect of physicochemical factors on spheroid formation and then evaluated the biological function of the prepared spheroids.

## 2. Materials and methods

### 2.1. Materials

Oleyl-*O*-poly(ethylene glycol)-succinyl *N*-hydroxy-succinimidyl ester (SUNBRIGHT® OE-080CS, mol. wt. 8525, purity >95%, polydispersity 1.02,  $n = 184$ ), diol PEG (SUNBRIGHT® DKH-20T, mol. wt. 19,684) and methoxy-PEG-OH (SUNBRIGHT® MEH-20T, mol. wt. 19,668) were obtained from NOF Corporation (Tokyo, Japan). All other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and were used without further purification.

### 2.2. Synthesis of crosslinker

A mixture of oleyl-*O*-poly(ethylene glycol)-succinyl *N*-hydroxy-succinimidyl ester (561 mg, 66  $\mu\text{mol}$ ) and ethylenediamine (2  $\mu\text{l}$ , 30  $\mu\text{mol}$ ) in *N,N'*-dimethylformamide (DMF) (4 ml) was stirred continuously at room temperature overnight. The solvent was then removed by evaporation under reduced pressure and the residue purified by dialysis against water. The resulting solution was lyophilized by freeze-drying to give a crosslinker (535 mg, 79% purity by gel permeation chromatography (GPC), 84% yield) in the form of a white fluffy material. The product was characterized with GPC (HLC-8220GPC system equipped with RI, TSK gel G4000H<sub>HR</sub> and TSK gel G3000H<sub>HR</sub> columns, Tosoh Corporation, Tokyo, Japan) in DMF (with 10 mM LiCl additive) and with Fourier-transform infrared spectroscopy (FTIR) (FTIR-8400S, Shimadzu Corporation, Kyoto, Japan). GPC:  $M_n = 17,585$ ,  $M_w = 18,042$ . IR (KBr,  $\text{cm}^{-1}$ ): 2889 (C–H of alkane), 1736 (C=O of ester), 1655 (C=O of amide), 1543 (N–H of amide), 1470 (C–H of  $-\text{CH}_2\text{O}-$ ), 1342 (C–H of alkane), 1281 (C–H of alkane), 1095 (C–C of alkane, C–O of ether). Critical micelle concentration (CMC) of the resulting crosslinker determined by pyrene method was 27 and 45  $\text{mg l}^{-1}$  at 25 and 37 °C, respectively, in phosphate-buffered saline (PBS).

### 2.3. Cell culture

The rat pancreatic  $\beta$ -cell line RIN, grown in RPMI-1640 medium (Sigma, MO, USA) containing 2% penicillin–streptomycin (Invitrogen Corporation, CA, USA) and 10% fetal bovine serum (FBS) (Invitrogen Corporation, CA, USA), was used for the experiment. Cells ( $1 \times 10^6$  or  $1 \times 10^5$  cells  $\text{ml}^{-1}$ ) were seeded onto a 96-well spheroid-plate with a non-adhesive surface and round bottom (Sumilon celltight® spheroid 96U plate, Sumitomo Bakelite co., Ltd., Tokyo, Japan) in 100  $\mu\text{l}$  of culture medium (with or without FBS supplemented) per well. Then the PEG crosslinker (sterilized under ultraviolet light for 15 min, 0.25, 2.5 or 25  $\text{mg ml}^{-1}$ ) in PBS was added to the 100  $\mu\text{l}$  portion per well. The cells were then cultured in a humidified incubator (ESPEC Corp., Osaka, Japan) at 37 °C in a 5%  $\text{CO}_2$  atmosphere. Half of the culture medium was replenished with new medium every third day. PEG and methoxy-PEG (mol. wt. 20,000) were also used as control materials. Cell growth was observed using an optical microscope (Olympus Corporation, Tokyo, Japan) with 4 $\times$  objective lenses and a camera.

### 2.4. Biochemical characterization

In order to evaluate the function of the spheroids, their insulin secretion and DNA content were measured. The medium was removed and RPMI-1640 medium containing 2  $\text{g l}^{-1}$  (11 mM) glucose without FBS was added. After incubation at 37 °C in 5%  $\text{CO}_2$  for 1 h, samples were centrifuged and the supernatant was preserved at  $-80$  °C until

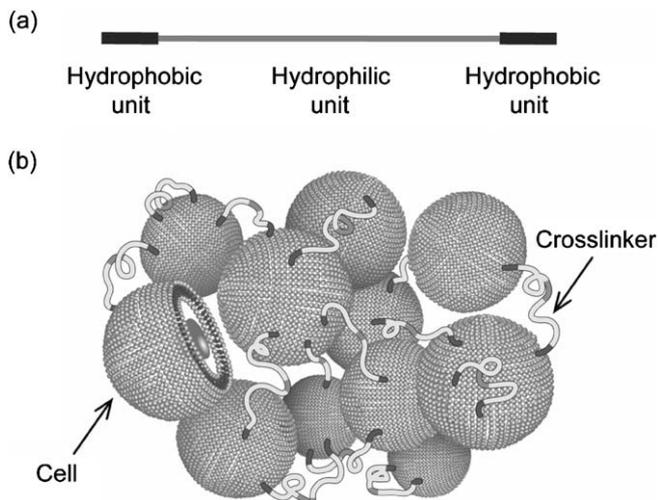


Fig. 1. Schematic illustration of (a) crosslinker and (b) crosslinking cells.

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