Synthesis and Evaluation of PEG Derivatives for Crosslinking Cells

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Summary: The objective of this study is synthesis, characterization, and evaluation of a novel crosslinker to form spheroids. Our approach was based on the crosslinking of cell membrane using a polymeric crosslinker via hydrophobic interaction. A crosslinker poly(ethylene glycol) derivative with oleyl groups as hydrophobic group at both ends was synthesized, and then characterized with gel permeation chromatography and Fourier-transform infrared spectroscopy. Furthermore, cell culture experiments were performed to confirm spheroid formation. Spheroids were successfully obtained when the crosslinker was added to cell suspension. This polymeric crosslinker was useful in the formation of cell spheroid.

Keywords: biomaterials; crosslinking; spheroid; tissue engineering

Introduction

Tissue engineering is an emerging technology that can be used to repair and regenerate damaged human tissue. The cells in a spheroid (multicellular mass) are known to possess enhanced functions compared with an individual cell[1,2] and therefore spheroid has great potential for its application in the field of tissue engineering. Recently, many researchers have performed to form spheroids using various techniques and materials.[3–5] However, special devices and techniques were required and it took very long time to form spheroid.

This paper reports on the development of a novel polymeric crosslinker that can enhance spheroid formation. The polymer has hydrophobic unit which can anchor to phospholipids bilayer of cell membrane; the polymer also has hydrophilic unit which promotes water solubility. We hypothesized that when this kind of polymeric crosslinker is added to cell suspensions, physical crosslinking would occur among cells via hydrophobic interaction. In this work, the synthesis, characterization, and evaluation of a novel crosslinker were performed to form cell spheroids.

Experimental Part

A crosslinker was prepared at room temperature by promoting reactions between ethylenediamine and poly(ethylene glycol) (PEG) oleyl ether with *N*-hydroxysuccinimide (NHS) at the end of PEG chain (NOF Co., Japan) in *N,N*-dimethyl formamide (DMF). The reaction product was purified via dialysis against water and lyophilized by freeze drying. The resulting product was then characterized with gel permeation chromatography (GPC) and Fourier-transform infrared spectroscopy (FT-IR).

Rat pancreatic β-cell line RIN was used for cell culture experiment. The cells were seeded on a 96-well spheroid-plate with non-adhesive surface and round bottom (Sumilon celltight® spheroid 96U plate, Sumitomo Bakelite Co., Ltd, Japan) in 100 μL culture medium RPMI-1640 (with or without FBS supplemented) per well. To this cell suspension, we added the crosslinker dissolved in PBS at various

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concentration with 100 μL per well. The incubation condition was fixed at 37 °C and 5% CO_2. Spheroid formation was observed using an optical microscope. PEG and methoxy PEG were also used as control materials.

Results and Discussion

The cell membrane is composed of a bilayer of amphiphilic phospholipids. Our approach was based on crosslinking of the cell membrane which is composed of phospholipid using polymeric crosslinker via the hydrophobic interaction. Poly(ethylene glycol) oleyl ether with *N*-hydroxysuccinimide was reacted with diamine in DMF at room temperature (Figure 1). Ethylenediamine was used as one of the typical diamines. Resulting crosslinker was then dialyzed against purified water. The average molecular weight of the product was confirmed by GPC in DMF. The molecular weight of the product (Mw 18,042) increased to approximately twice that of the starting PEG derivative (Mw 8,525). The FT-IR spectrum of the product showed the existence of absorption bands assigned to amide groups (C=O, 1655 cm^{-1}); it also showed a new peak characteristic of the N–H of the amide groups at 1543 cm^{-1}. Results from GPC and FT-IR indicated that the PEG derivatives were successfully introduced into both ends of diamine in one step.

Using the obtained crosslinker, cell culture experiments were performed to confirm the spheroid formation. The effects of incubation time, crosslinker concentration, serum, and number of cells for formation of spheroids were evaluated. The polymer concentration was varied from 0 to 25 mg/mL. Figure 2 shows the effect of polymeric crosslinker on spheroid formation. The spheroid formation was promoted in the presence of the crosslinker. After 3 days, spheroids with large size were observed when the polymeric crosslinker concentration was 2.5 mg/mL. We also observed that the size of spheroids decreased with increasing crosslinker concentration. As the cell is mutually adjacent by using of the crosslinker, it may become easy to form spheroid through the cell adhesion factor. Because the density of spheroid rises by constructing bridges with a lot of crosslinker, the small size spheroid was made.

The effect of serum on spheroids was also evaluated. The spheroids with smaller size were obtained when cells were cultured in a medium without serum. These results suggested that some of the lipids or proteins

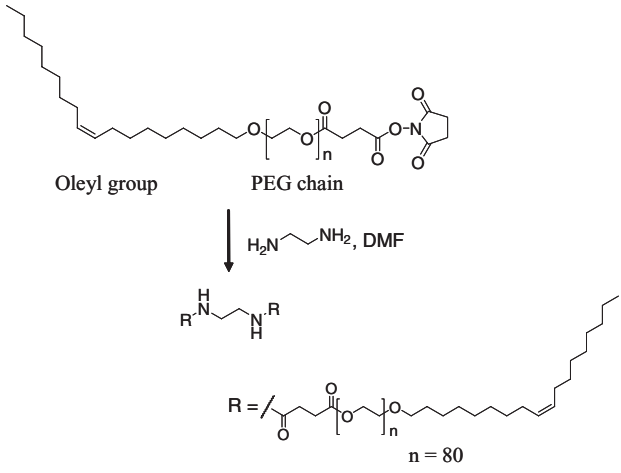

Figure 1.
Synthetic scheme of crosslinker.

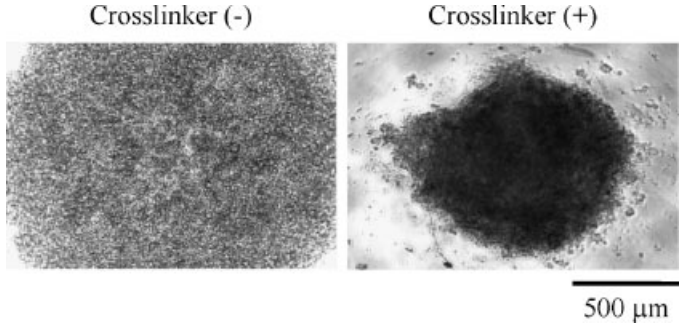

Figure 2.
Photographs of cell cultured without (left) and with (right) crosslinker (25 mg/mL).

in serum suppress the physical crosslinking between cells. Cell number also affected the size of resulting spheroids. When the crosslinker was applied to a large number of cells, spheroids with large size were formed. It was shown that the size of the resulting spheroids could be controlled by changing the initial number of cells. No difference in this behavior was observed in the cells, with or without serum. On the other hand, the cell spheroids were not formed when PEG and methoxy PEG (Mw 20,000) were applied. These results suggested that the cell spheroids with this polymeric crosslinker were formed by anchoring cells via hydrophobic interaction.

Conclusions

In this work, a novel polymeric crosslinker which promotes spheroid formation was developed. At low crosslinker concentration, spheroids with large size were obtained. On the other hand, spheroids with smaller size were formed at high crosslinker concentration. At low crosslinker concentration, the formation of spheroids occurred slowly, and the aggregation became small with increasing culture time. In contrast, spheroid formation was rapid when the crosslinker concentration was high. These spheroids also became small with time. Under the same crosslinker concentration, medium without serum proved to be a favorable condition for promoting the formation of spheroids. On the other hand, the size of obtained spheroids increased with increasing initial cell number. This anchoring technique seemed quite promising for cell spheroid formation.

Acknowledgements: This work was financially supported in part by the Coordination Fund for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Development of artificial organs utilizing with nanotechnology and materials science).

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