# Synthesis and Evaluation of Polymer for Crosslinking Cells

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## Introduction

Tissue engineering is important and alternative approach to repair and regenerate damaged human tissue. The cells in spheroid (multicellular mass) possess enhanced functions compared with the individual cell. Therefore, spheroid has great potential to apply for tissue engineering. Spheroid formation was performed by many researches using some technologies and the materials. Those researchers have succeeded in the formation of spheroid, however, these need special devices and the technologies. In addition, it takes much time to form spheroid.

The objective of this study is to develop the novel polymeric crosslinkers to enhance cell aggregation. In order to accomplish this objective, a novel polymeric crosslinker was designed. This polymer has hydrophobic unit which can anchor to phospholipids bilayer of cell membrane. In this polymer, hydrophilic unit promotes water solubility. We hypothesized that when this kind of polymeric crosslinker was just added to cell suspensions, physical crosslinking may occur between cells via hydrophobic interaction. In this study, the effect of crosslinker concentration, incubation time and serum for formation of cell aggregation was evaluated.

## Experimental

The crosslinker was prepared by the reaction between ethylenediamine and oleyl poly(ethylene glycol) (PEG) ether with *N*-hydroxysuccinimide (NHS) at the end of PEG chain (NOF Co., Japan) in *N,N*-dimethyl formamide (DMF) at room temperature. The resulting product was then characterized with gel permeation chromatography (GPC) and Fourie-transformation infrared resonance (FT-IR).

Rat pancreatic β-cell line RIN was used for cell culture experiment. The cells were seeded on 96-well spheroid-plate which has a non-adhesive surface and round bottom (Sumilon celltight® spheroid 96U plate, Sumitomo Bakelite Co., Ltd, Japan) in 100 μL culture medium (with or without FBS supplemented) per well. Then, the crosslinker in PBS was added with 100 μL portion per well at various concentrations. Incubation condition was fixed at 37 ˚C and 5% $CO_2$. PEG and Methoxy PEG were also used as control materials.

## Results and discussion

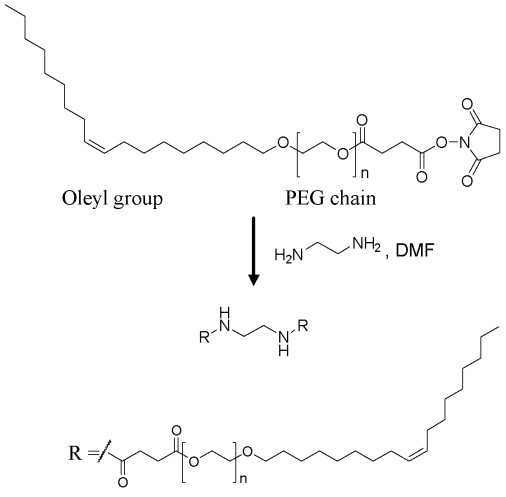


**Figure 1.** Synthetic scheme of crosslinker.

The cell membrane is composed of a bilayer of amphiphilic phospholipids. Our approach to bridge cells is based on the crosslinking of the cell membrane which is composed of phospholipid via the hydrophobic interaction.

Poly(ethylene glycol) oleyl ether with *N*-hydroxysuccinimide was reacted with diamine in *N,N*-dimethyl formamide 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 $cm^{-1}$), and the appearance of 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 introduced to the both ends of diamine at one step.

Using the obtained crosslinker, cell culture experiment was performed to confirm spheroid formation. The polymer concentration was varied from 0 to 25 mg/mL. After 3 days, cell aggregation with large size was observed when the polymer concentration was 2.5 mg/mL. The size of cell aggregation decreased with increasing crosslinker concentration. When the crosslinker was applied to the large number of cell, cell aggregation with large size was formed.

The effect of serum on cell aggregation was also evaluated. The cell aggregation with smaller size was obtained when cells were cultured in the medium without serum. These results suggested that the some lipids or proteins in serum suppress the physical crosslinking between cells. Cell number also affected the resulting cell aggregation. When the crosslinker was applied to the large number of cells, cell aggregation with large size was formed. No difference of this behavior was observed in the cells with or without serum. On the other hand, the cell spheroid was not formed when PEG and Methoxy PEG were applied. These results suggested that the cell assembly with the crosslinking agent was occurred by hydrophobic groups in the crosslinker. Furthermore, we also performed biochemical analysis of resulting spheroids.

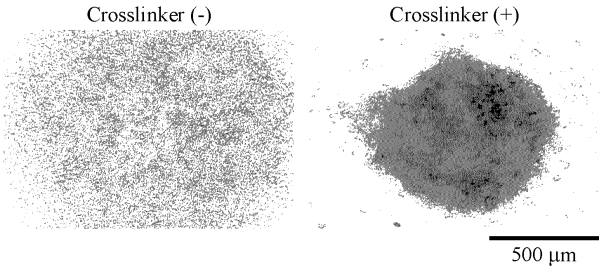


**Figure 2.** Photographs of cell cultured without (left) and with (right) crosslinker.

## Conclusions

The novel crosslinker which promotes spheroid formation was developed. At the low crosslinker concentration, cell aggregation with large size was obtained. On the other hand, at the high crosslinker concentration, cell aggregation with smaller size was formed. At low crosslinker concentration, cell aggregation was slowly occurred and the aggregation became small with increasing culture time. In contrast cell aggregation rapidly occurred when the crosslinker concentration was high level. These aggregations also became small with time. Under the same crosslinker concentration, medium without serum is the favorable condition to promote cell aggregation. On the other hand, the size of obtained cell aggregation increased with increasing initial cell number.

## Acknowledgments

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