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Combination of two different molecular weight methoxy polyethylene glycols for camouflaging of pancreatic islets from immune response

Aghajani-Lazarjani H.[1,2], Vasheghani-Farahani E. [2], Hashemi-Najafabadi S. [2], Shojaosadati S. A. [2]
[1]Pathology & Medical Biol, Groningen, The Netherlands. [2]Chem Eng, Tarbiat Modares Univ, Tehran, Iran
* E.Vasheghani-Farahani , evf@modares.ac.ir

INTRODUCTION

Islet transplantation has been appeared as a promising therapy for diabetes (Shapiro, 2006). However, clinical application of islets is limited due to rejection of transplanted cells and side effect of immunosuppressive drugs. Enormous researches have led to development of other techniques for protecting islets such as grafting polyethylene glycol to the surface of islets (Barani 2010). PEG is a highly hydrated polymer with flexible chains due to repeating highly mobile ether units. The hypothesis of PEG conjugation, PEGylation, is that the conjugated PEG molecules protect islets from immune cell's attack because of its low interfacial free energy with water, unique properties in aqueous solutions, high surface mobility, and substantial steric stabilization effects (Hershfield 1997, Scott 1997). Based on the promising findings on red blood cell (RBC) PEGylation, this approach were applied to islets without compromising viability and functionality (Hashemi-Najafabadi 2006, Aghajani-Lazrajani 2010).

Fisher showed that PEG with higher molecular weight inhibited RBC-RBC adhesive interactions and led to very effective reduction of low-shear blood viscosity , anti-aggregant, and immunologically masked (Fisher 2001). By considering this finding, the effect of two different molecular weights of PEG and their combination on the *in vitro* activation of immune islet cells was investigated.

MATERIALS AND METHODS

Pancreatic islets were isolated from male Wistar rats weighing 250-300 g by the collaganase digestion method of Lacy and Kostianovsky(Lacy 1967). After handpicking, islets were cultured overnight in RPMI-1640 medium containing 10% fetal bovine albumin (FBS) at 37°C in humidified atmosphere of 5% CO_2 in air. To graft PEG onto capsule of islets, the isolated islet were washed twice with HBSS(pH 7.4), followed by suspending in 10mL HBSS containing mPEG-SC according to Table 1. Islet incubated in humidified atmosphere of 5% CO_2 in air for 1h. The co-culture of PEGylated and free islets (30 islet/well) with lymphocyte (5×10^5) isolated by ficoll density gradients method was performed in 96-well plate. The culture medium was sampled at 3, 5 and 7 days and measured by IL-2 kit. The viability of islets was determined by flourometric method using acridine orange (AO) and propidium iodide (PI). The insulin secretion stimulated by glucose was measured in order to evaluate the functional activity of islets. 8 islets were incubated at 37°C for 60min in 1mL Krebs-Ringer bicarbonate buffer (KRBB) containing low concentration (2.8mM) (basal secretion) and high concentration of glucose (16.7mM) under a humidified air of 5% CO_2 at 37°C. Samples were measured by insulin radioimmunoassay (RIA).A non-parametric F-test was used to evaluate the significant difference between the means. Values are expressed as the mean± SE.

Table 1 : Concentration and molecular weight of mPEG-SC

Exp	Concentration (mg/mL)	Molecular Weight (kDa)
1	22	5
2	22	10
3	13.2(10 kDa)+ 8.8(5kDa)	8.08 (10+5)

RESULTS AND DISCUSSION

Functional activity of the islets grafted with 5 and 10kDa PEG in static culture did not show any significant difference with respect to cultured free islets in terms of insulin secretion. As shown in Fig.1 free islets insulin secretion was 7.62±1.86 µg/L and insulin secretion by islets coated with 5 and 10kDa mPEG-SC was 6.73±2.67µg/L, and 6.47±1.79µg/L, respectively.

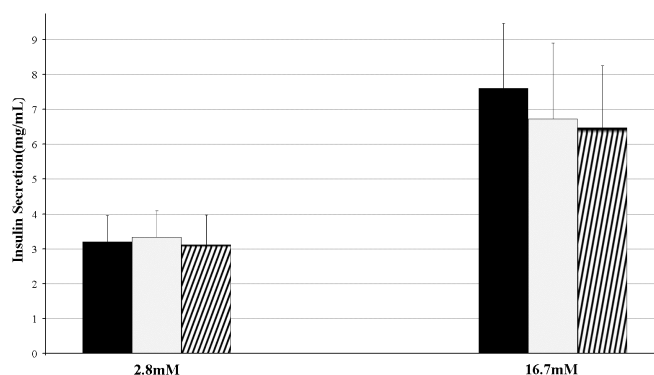

Fig 1. Secretion of insulin from islets in static culture; (■) free islets, (□) PEG (5kDa)-grafted islets, and (▨) PEG (10kDa)-grafted islets. Data presented as mean± SE, n=3.

After 5 days of co-culturing free islets with lymphocyte, free islets were destroyed and the integrity of their capsule completely lost. The viability of free islets was 8.6±1.2% after 7 days of co-culturing (Fig.2). While for PEG-grafted islets, the morphology did not change by co-culturing. The Viability of PEG-grafted islets maintained

at 75.34±4.96 and 80.57±1.2, respectively for islets PEGylated by 5 kDa and 10kDa mPEG-SC at seventh day.

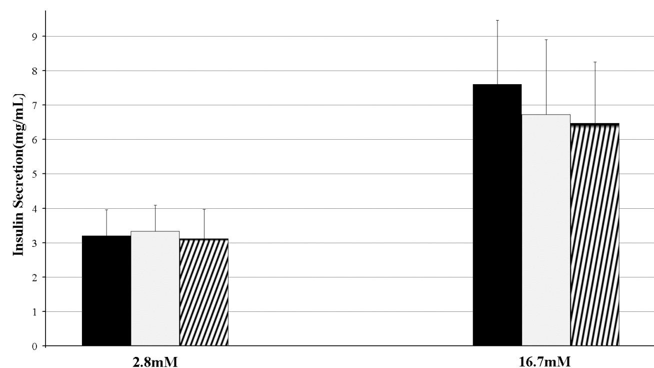

Fig 2. Viability of islets at first (A) and 7(B) days of co-culturing; (■) free islets, (□) PEG (5kDa)-grafted islets, and (▨) PEG (10 kDa)-grafted islets. Data presented as mean± SE, n=10.

The concentration of secreted IL-2 for free islets co-cultured with lymphocytes was two times greater than that for PEG-grafted islets (257.47±10.28 pg/mL). However, Lymphocytes cultured with islets grafted by 5 kDa mPEG-SC secreted larger amount of IL-2 (151.97±16.26 pg/mL) compared to the secreted IL-2 in the presence of islets coated with 10kDa mPEG-SC (128.13±11.81 pg/mL). At the same time, the amount of IL-2 secreted in the presence of islets coated with 2/3 combination of 5 and 10kDa MPEGs was the lowest (78.52± 15.34 pg/mL). It is postulated that mPEG with higher molecular weight may be more suitable for surface coating of cells due to its high shielding effect. The steric exclusion effect of the grafted polymer chains primarily prevents protein adsorption (Le, 2010). This effect maximized when chains are grafted at higher density, i.e. with small separation between chains. But the high density grafting is difficult to achieve with polymers which have a large gyration radius. When long and short chain polymers are attached onto surface together, higher density can be achieved. Therefore, this effect can explain why the minimum point occurred at 8.08 kDa.

CONCLUSION

Increasing molecular weight of mPEG-SC from 5 to 10 kDa for PEGylation of pancreatic Islets caused an initial decrease in IL-2 secretion with a minimum point occured at 8.08 kDa which corresponds to 40-60% combination of 5 and 10 kDa.

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