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Biomaterials 26 (2005) 403–412
Biomaterials
www.elsevier.com/locate/biomaterials

Cytoprotection of PEG-modified adult porcine pancreatic islets for improved xenotransplantation

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Received 12 January 2004; accepted 19 February 2004

Abstract

Functional poly(ethylene glycol) (PEG) derivatives, including monosuccinimidyl PEG (MSPEG) with molecular weight (MW) of 2000 (2 kDa) as well as 5 kDa and disuccinimidyl PEG (DSPEG) with MW of 3 and 6 kDa, were synthesized and characterized. They were used to modify the surface of adult porcine islets for cytoprotection. The islets were isolated, purified and modified with functional PEG. Untreated porcine islets were used as control. An in vitro human antibody/complement-mediated cytotoxicity test based on the release of intracellular lactate dehydrogenase was used to evaluate cytotoxicity of human serum to the modified islets. In vitro cell viability was assessed using membrane-integrity straining and islet metabolism in culture. In vitro islet functionality was evaluated by glucose-stimulated insulin release of islets in static incubation with human serum. In vivo islet functionality was evaluated by monitoring non-fasting blood glucose level in streptozotocin-induced diabetic (SCID) immunocompromized mice after intraportal transplantation of porcine islets. Results show that all the PEG derivatives used in the study showed significant in vitro and in vivo cytoprotections against cytotoxic effects elicited by human serum and diabetic SCID mice, respectively, to porcine islets. DSPEG derivatives combined with human albumin exhibited a better cytoprotection, as compared to MSPEG ones, due to the capacity of the succinimidyl groups to selectively react with amino groups of the albumin under physiological conditions. The effects of both MW and concentration of the PEG derivatives on cytoprotection were significant. It appears that this novel biotechnology will be an attractive approach for improved xenotransplantation of islets.

Keywords: PEG derivatives; Synthesis; Characterization; Xenotransplantation; Cytoprotection; Antibody/complement-mediated cytotoxicity; Cell viability; In vitron and in vivo islet functionality

1. Introduction

Type I diabetes mellitus, also known as insulin-dependent diabetes mellitus, is an autoimmune disease wherein pancreatic islets of Langerhans are destroyed. Type I diabetes affects over 15.7 million people in the United States, a number predicted to increase to as many as 25 million by 2010 [1]. Patients with type I diabetes can no longer produce insulin in response to glucose in their diet because insulin is synthesized in the islets. Current therapy for patients with type I diabetes includes insulin injections, dietary constraints, and exercise. However, insulin therapy cannot duplicate a normal physiological response and thus diabetics experience an increased incidence of heart disease, nephropathy, and neuropathy [2]. Severe complications of the disease have prompted other types of treatments to be investigated, including transplantation of the entire pancreas or of purified islet preparations (cell therapy). However, the morbidity of surgery and the chronic immunosuppression that accompanies transplantation must be weighed against the potential benefit of improved glucose metabolism. Usually, this option is not considered unless another transplant is required at the same time (e.g., a simultaneous kidney transplant [3]). The desire to transplant islet tissue without the need

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doi:10.1016/j.biomaterials.2004.02.048

for immunosuppression has led to development of immunoisolation devices where islets might be isolated from the host's immune system by barriers or membranes permeable to low molecular weight (MW) species such as glucose and insulin but impermeable to high MW immune proteins such as immunoglobulins M (Ig M) and G (Ig G) as well as other complement cytotoxins.

Although allogenic islet transplantation is clinically effective for type I diabetic patients via immunosuppressive agents, the future for allografts seems unclear because of donor scarcity [4]. As an alternative, xenogeneic pancreatic islets are strong candidates for islet transplantation. In this regard, pigs are an attractive source of islets because they breed rapidly and there exists a long history of porcine insulin use in humans as well as the potential for genetic engineering. Although the potential for infection of recipients with xenogeneic agents and the risk of transmission to the general population, particularly, infection with porcine endogenous retrovirus in human, are major concerns, recent research has shown that natural xenoreactive antibodies can prevent the infection [5,6]. In addition to it, several immunological obstacles, however, must also be overcome, in particular, the susceptibility of porcine pancreatic islets to destruction by immunological processes and exposure to human blood. It is known that antibodies (or immunoglobulins) and complement system destroy cells via abundant surface antigens on the transplanted cells [7]. These surface antigens are mainly composed of oligosaccharides on glycolipids and glycoproteins (Fig. 1(A)), which are solely responsible for antigen–antibody and complement-mediated reactions [7]. Recent advances in biotechnology suggest that there are some scientific strategies that can prevent the immune response induced by foreign cells or xenogens [8], which include microencapsulation of cells [9–22] and surface modifications [23–29].

Many immunoglobins or antibodies and components of the complement system exist in blood serum. IgG, IgM, IgA, IgD and IgE are the most common immunoglobins [8]. The complement system comprises a group of more than 30 serum and cell surface proteins with MWs in the range between 25 and 750 kDa. Both immunoglobulins and the complement system are responsible for cytotoxicity to transplanted cells and tissues. Islets or tissues can be rejected via action of both antibodies and antibody-activated complement systems [8].

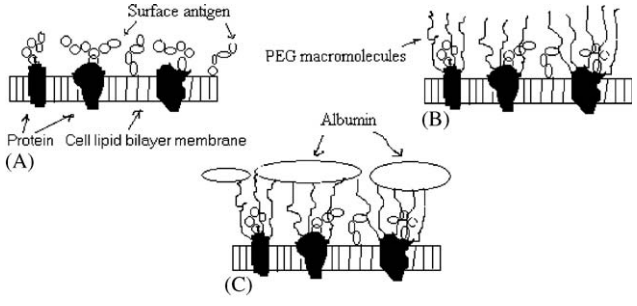

Fig. 1. Schematic diagram illustrating cell surface modification of islets using PEG and Albumin: (A) Cell membrane containing surface antigens (oligosaccharide) that cause immune reactions, proteins and lipid bilayers; (B) PEG grafted cell surface for cytoprotection against antibodies (IgM and IgG); and (C) PEG combined with albumin for better cytoprotection.

To protect islets from immune-mediated destruction, camouflaging the surface of islets is necessary for immunoisolation and immunoprotection. Two major approaches have been tried so far to prevent immunogenic reactions on the cellular surface. One is microencapsulation of the cells [9–22] and the other is surface modification of the cells [23–29]. The strategy in the former is very similar to those applied in drug delivery. Synthetic poly(vinyl alcohol) [11,12], poly(lactide-*co*-glycolide) [13], dimethylaminoethyl methacrylate-methyl methacrylate copolymer [14], natural and biodegradable polymer alginate with or without polylysine [4,7,10,15–19] and natural agarose [20,21] have been used for encapsulation of pancreatic islets. Microcapsulation of islets by a polylysine–alginate polymer complex is the most successful example of this technology [9]. The formed semi-permeable membrane permits nutrient flow and oxygen transport but prevents immunogenic reactions. However, pitfalls to microencapsulation include reduced lifespan of the cells due to polymer biodegradation, permeability of the capsules, fragility, limited surface areas, etc. [22].

Poly(ethylene glycol) (PEG) has been successfully used to reduce plasma protein adsorption and platelet adhesion in making blood compatible vessels and devices or surface modification of the blood compatible polymers due to its low interfacial free energy with water, unique solution properties in aqueous solution, high surface mobility, and steric stabilization effects [30,31].

Surface modification studies of the cells have been given to several cells including red blood cell, white blood cell, islet cell, etc. Most studies have been focused on red blood cells. Modification of the surface of the red cell using PEG originated from the concept that PEG modified proteins and enzymes render them non-immunogenic [32,33]. Applying the same concept, Jeong and Byun [23] first used a non-immunogenic PEG-cyanuric chloride to modify the surface of red cells and found decreased agglutination and antibody binding after the modification. The morphology of the cells remained intact. Murad et al. [24,25], Scott and Murad [26] applied the same strategy to anchor PEG-cyanuric chloride onto both red cells and T lymphocytes and likewise found that both morphology and biological function of the cells did not change but their immunogeneicity dramatically dropped. Hortin et al. [27] and Hortin and Huang [28] modified mouse red cells using a