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Review

A hybrid of cells and pancreatic islets toward a new bioartificial pancreas

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ABSTRACT

Cell surface engineering using single-stranded DNA–poly(ethylene glycol)-conjugated phospholipid (ssDNA–PEG-lipid) is useful for inducing cell–cell attachment two and three dimensionally. In this review, we summarize our recent techniques for cell surface engineering and their applications to islet transplantation. Because any DNA sequence can be immobilized onto the cell surface by hydrophobic interactions between ssDNA–PEG-lipid and the cellular membrane without impairing cell function, a cell–cell hybrid can be formed through the DNA hybridization. With this technique, it would be possible to create three-dimensional hybrid structures of pancreatic islets coated with various accessory cells, such as patients' own cells, mesenchymal and adipose-derived stem cells, endothelial progenitor cells, neural crest stem cells or regulatory T cells, which might significantly improve the outcome of islet transplantation in diabetic patients.

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1. Introduction

Diabetes is characterized by hyperglycemia due to an absolute or relative lack of insulin to cover the metabolic needs of the body. The disease is commonly divided in type 1 and type 2 diabetes but its etiology and pathogenesis is quite heterogeneous. A common denominator is, however, the loss of functional insulin producing cell (beta-cell) mass. This is caused in by immunological mechanisms in type 1 diabetes and is probably inherent when exposed to external stress in type 2 diabetes. Exogenous insulin therapy cannot

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Abbreviations: PEG-lipid, poly(ethylene glycol)-conjugated phospholipid; IBMIR, instant blood-mediated inflammatory reaction; islets, islets of Langerhans; PMPC, poly(2-methacryloyloxyethyl phosphorylcholine).
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approximate normal physiological pulsatile insulin secretory patterns with complete integrity and rarely attains normal blood glucose levels without the risk of major hypoglycemic episodes and devastating complications including retinopathy, nephropathy, and neuropathy; therefore, more effective therapy needs to be established.

Presently, diabetes can neither be prevented nor cured by other means that cell replacement including pancreas and pancreatic islet transplantation. Islets are aggregates of 1000–2000 endocrine cells (including beta-cells) that form cell clusters of up to 300 μm within the pancreas. For clinical islet transplantation, these cells are isolated from the pancreases of a few brain-dead donors and infused into the liver via the portal vein of diabetic recipients or their body. Because the procedure is less invasive to patients, this treatment is very promising, and various related clinical reports have been published since the beginning of the 1970s [1–3]. However, recipients must take immune-suppressive drugs to protect grafts from immune rejection.

In addition, the first days after transplantation are characterized by dynamic changes resulting in substantial early cell death and dysfunction due to multiple factors including insufficient graft revascularization [4], and re-innervation [5], alloimmune rejection and recurrence or persistence of autoimmunity [6], toxicity of immunosuppressive regimens [7], liver ischemia with subsequent cytotoxicity [8] and inflammatory reactions. Exposure of the islet surface to recipient blood activates blood coagulation and a complement response, which subsequently induces inflammation after infusion into the liver [9–11]. This series of reactions, is recognized as instant blood-mediated inflammatory reaction (IBMIR), leads to immediate islet destruction immediately after intraportal transplantation [12]. Despite intense scientific efforts, this issue still remains unresolved in clinical islet transplantation. Several studies have been conducted to examine ways to protect islets from IBMIR using systemic administration of anticoagulants, anti-thrombin inhibitors, melagatran [13], low-molecular weight dextran sulfate [14], and some complement inhibitors [9,15]. However, systemic administration is always associated with a bleeding risk. Alternatively, our group has examined immobilization of bioactive substances and living functional cells onto the islet surface, which could provide local regulation of unfavorable reactions [16–20]. By using this technique the risk of bleeding, associated with systemic modulation of coagulation and complement after intraportal islet transplantation would be avoided. In preclinical studies, co-transplantation of islets of Langerhans with accessory non-islet cells, such as mesenchymal and adipose-derived stem cells [21–24], endothelial progenitor cells [25–27], neural crest stem cells [28,29] or regulatory T cells [30], has been show to improve the outcome of islet transplantation. Thanks to their pleiotropic effects, including angiogenic, anti-apoptotic and immunomodulatory effects, these cells might prove to be superior compared to drug-based approaches that often target single components of islet graft failure. In particular, our group has already shown that coating of the islet surface with endothelial cells has the potential to significantly inhibit IBMIR completely because endothelial cells express regulators for coagulation and complement systems and the exposed surface can mimic the endothelium of the recipient. In fact, our group has already published some promising results [31].

Co-transplantation of islets and other cells thus can be an alternative to the surface-modification approach. However, the hybrid of islets and other cells is not easy to achieve because cell–cell attachment cannot be induced without general cadherin–cadherin interactions. Although interaction with collagen on the islet surface can be available for attaching endothelial cells, an engineering approach should be established to expand this idea to the use of various functional cells. To address this issue, we have used a cell surface-modification technique with single-stranded DNA–PEG-conjugated phospholipid (ssDNA–PEG-lipid), which enabled us to induce cell–cell attachment two dimensionally (2D) and three dimensionally (3D). In this review, we summarize our recent techniques for cell surface engineering and their applications to islets transplantation.

2. Immobilization of ssDNA on the cell surface by hydrophobic interactions

ssDNA can be immobilized on the living cell surface without influencing cell viability by using an amphiphilic polymer, PEG-conjugated phospholipid (PEG-lipid), which consists of both a hydrophilic domain (PEG) and a hydrophobic domain (lipid) (Fig. 1A) [19,32,33]. For this purpose, ssDNA–PEG-lipid is used where any sequence of DNA is available for the conjugation [34]. When the lipid domain of ssDNA–PEG-lipids is spontaneously incorporated into the lipid bilayer membrane by hydrophobic interactions, the hydrophilic ssDNA–PEG domain is displayed on the cell surface (Fig. 1B). Here the molecular weight of PEG of ssDNA–PEG-lipids was 5 kD. The role of the PEG is a spacer for anchoring ssDNA on cell surface. Thus, it is possible to immobilize ssDNA on the cell surface. Although ssDNA–PEG-lipids are incorporated into the cellular membrane, there was no cytotoxicity for primary cells, cell lines, and islets after the cell surface modification. In addition, this surface modification of islets with ssDNA–PEG-lipids does not impair insulin secretion ability from the insulin release assay [20]. These results indicated that our approach with ssDNA–PEG-lipids did not influence cellular function.

One approach is the use of polyA20–PEG-lipid and polyT20–PEG-lipid for attaching different cells through DNA hybridization. Because the hybridization between polyA20 and polyT20 is a rapid and specific reaction, it is easy to design 2D and 3D cell organization. Addition of ssDNA–PEG-lipid solution to the cell suspension and incubation at room temperature for 30 min leads to modification of the cell surface with ssDNA–PEG-lipid. To examine the existence of ssDNA (i.e., polyA20) on the cell surface, FITC-labeled complementary ssDNA′ (FITC-polyT20) is used (Fig. 1C). Clear fluorescence from FITC-polyT20 is observed only on the cell surface, which is treated with polyA20–PEG-lipid, indicating the immobilization of polyA20–PEG-lipid. Of importance, the whole cell surface is uniformly covered with polyA20–PEG-lipid. Actually, there are lots of membrane proteins existing on cellular membranes. Since ssDNA–PEG-lipids are hydrophobically interactive with lipid bilayer membrane domains, they are separately located on the cell membrane and available for DNA hybridization. Therefore, using ssDNA–PEG-lipid makes it possible to immobilize any DNA sequence on the cell surface for further reactions that lead to 2D and 3D cell organization. Multiple membrane proteins and glycocalyx components such as glycoproteins and glycolipids cover the cell membrane. The combined thickness of this layer is assumed to be up to several hundred nanometers. When the cell membrane is modified with ssDNA–PEG-lipids with 5 kD of PEG, they are surrounded with membrane proteins and glycocalyx, where ssDNAs are presumably located at the lower position than various membrane proteins. However, ssDNA molecules on the cell membrane can be accessible to the complementary DNAs when they are added. Flexible PEG chain might be useful for the reaction.

3. 2D cell alignment by ssDNA–PEG-lipid

To align cells on the substrate in a patterned way, various approaches have been reported. Usually, extracellular matrix such as fibronectin, vitronectin, or RGD peptide is immobilized onto the specific area, which allows cells to adhere selectively. Although the