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Size-controlled insulin-secreting cell clusters

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ABSTRACT

The search for an effective cure for type I diabetes from the transplantation of encapsulated pancreatic β-cell clusters has so far produced sub-optimal clinical outcomes. Previous efforts have not controlled the size of transplanted clusters, a parameter implicated in affecting long-term viability and the secretion of therapeutically sufficient insulin. Here we demonstrate a method based on covalent attachment of patterned laminin for fabricating uniformly size-controlled insulin-secreting cell clusters. We show that cluster size within the range 40–120 μm in diameter affects a variety of therapeutically relevant cellular responses including insulin expression, content and secretion. Our studies elucidate two size-dependent phenomena: (1) as the cluster size increases from 40 μm to 60 μm, glucose stimulation results in a greater amount of insulin produced per cell; and (2) as the cluster size increases beyond 60 μm, sustained glucose stimulation results in a greater amount of insulin secreted per cell. Our study describes a method for producing uniformly sized insulin-secreting cell clusters, and since larger cluster sizes risk nutrient availability limitations, our data suggest that 100–120 μm clusters may provide optimal viability and efficacy for encapsulated β-cell transplants as a treatment for type I diabetes and that further in vivo evaluation is warranted.

1. Introduction

The development of a bio artificial pancreas began in 1933 when tissue containing insulin-secreting cells was first transplanted as a potential diabetes treatment [1]. Nearly 80 years later, human trials currently underway in New Zealand evaluating encapsulated islet transplants without immunosuppression report significant reductions in hypoglycemic events, but have yet to achieve reliable insulin independence. Transplantations of unencapsulated human cadaveric β-cells containing islets are currently available and provide at least one year of insulin independence for 80% of recipients [2]. While these pancreatic β-cells are able to sense glucose and secrete insulin at the appropriate level needed for glucose homeostasis, debilitating immunosuppression is required [3] and the availability of cadaveric islets is extremely limited [4]. Significant advances in encapsulation technologies over the past several decades promise to obviate the need for immunosuppression [5,6]. Additionally, animal sources [7,8] and human stem cell sources [9,10] are being cultivated to overcome supply limitations. While these developments promise to overcome some of the limitations preventing wide-scale adoption of this therapeutic approach, efforts to control the size of transplanted clusters have been lacking.

Two independent size requirements must be satisfied in order to achieve viable islet transplants with sufficient insulin secretion. First, very small clusters do not exhibit therapeutically appropriate insulin secretion because of its dependence on sufficient cell–cell contact. For example, pancreatic β-cell pairs and monolayers secrete greater insulin per cell after glucose stimulation than isolated β-cells [11,12]. Furthermore, glucose-dependent calcium oscillations, a characteristic of appropriately functioning islets, occur more frequently in cell clusters compared with isolated cells [13]. Second, excessively large clusters suffer from nutrient availability limitations. Relying solely on passive diffusion, oxygen and nutrient requirements are attained only when cells are within 100–200 μm from a capillary [14–16]. In fact, necrosis has been observed on the inside of large isolated islets [17,18]. As expected from these results, islets smaller than 150 μm exhibit improved insulin secretion and viability in clinical studies than larger islets [19]. While cell encapsulation in a material with pore sizes small enough to inhibit the passage of antibodies protects transplants from the immune response [20], the same material also inhibits the growth of new blood vessels and prevents access to perfusion that is essential for nutrient availability throughout large islets in the native pancreas [21]. Despite significant evidence supporting

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