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Nanoencapsulation enhances graft survival and function in diabetic mice of islet transplantation

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INTRODUCTION AND OBJECTIVES

Allogeneic islet transplantation is currently considered by many the most promising method for curing Type 1 diabetes (Shapiro 2000). Nevertheless, it is limited by the post-transplantation loss of functional islets through deleterious responses from the complex host immune system. Immunoisolation through encapsulation is effective in maintaining long-term function in the transplanted islets without undesired immunosuppression (Lim 1980; Teramura 2010). Among different encapsulation technologies, conformal nanocoating has the potential to offer the biological benefits of conventional macro- and micro-type approaches with the added value of greatly reduced transplant size enabling implantation into any site suitable for naked islets (Teramura 2010; Wilson 2008).
In the current study, we have adapted a layer-by-layer deposition technology to buildup conformal nanocoating for mouse pancreatic islets which covers individual cells in nanolayers, achieving effective encapsulation without significantly increasing the size of the islet. The aim of the study was to improve islet transplantation outcomes by maintaining appropriate secretory function whilst preventing the instant blood-mediated inflammatory reaction and the host-graft interactions involved in host-versus-graft allogeneic immune rejection.

MATERIALS AND METHODS

The nanoencapsulation approach uses alternative deposition of layers of charged linear polysaccharides as the coating materials, as shown in Fig 1. This process was repeated until a layering scheme of cells/(chitosan-PC/alginate)n is produced, where n represents the number of bilayers. The polysaccharides used in the study contains at least one charge in each monosacchride unit which serves as the binding site, offering hundreds of binding sites for each polysaccharide molecule, thus providing a very strong affinity to the charged cell surfaces. Multilayer film formation is possible because of charge reversal on the film surface after each adsorption step.
We used streptozotocin-induced Type 1 diabetes mouse islet transplantation model to assess the effectiveness of nanoencapsulated islets in maintaining blood glucose after implanted under kidney capsule of the animal.

RESULTS AND DISCUSSION

Fluorescence and TEM studies of the nanoencapsulation of mouse islets. The deposition of multilayers was assessed by fluorescence microscopy following the incorporation of a layer of poly-L-lysine labelled with Alexa Fluor 647 into the outer layer as a fluorescent marker. As shown in Fig 2, the addition of the fluorescent layer to the islets resulted in strong fluorescence localized mainly on the face of islet cells at the islet periphery, which is consistent with the extralellular architecture of the islets, indicating complete coverage.

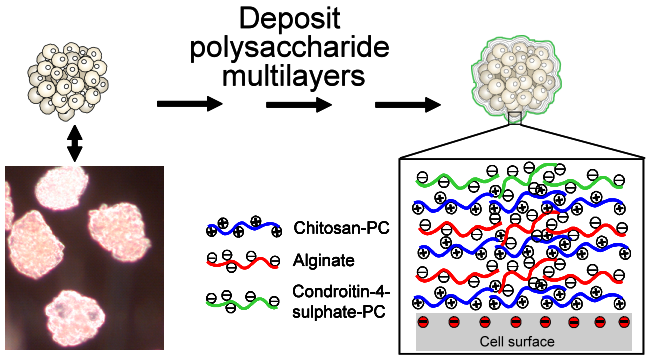

Fig. 1. Schematic representation of nanoencapsulation of the pancreatic islets: layer-by-layer deposition of charged polysaccharides on islet surfaces.

The deposition of the polysaccharide multilayers on the individual islets was further confirmed by high-resolution TEM imaging of the sectioned encapsulated islets, as shown in Fig 2. The image of the ultrastructure showed the intact coating multilayers consisting of nanolayers of wet thickness around 80 nm covering the outer surface of the islets. Meanwhile, the insulin secreting vesicles were aligned along the plasma membrane ready for exocytosis, suggesting the beta cells were healthy and unaffected by the coating.

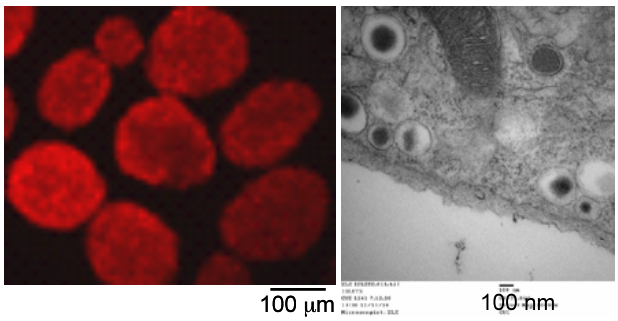

Fig 2. Confirmation of nanoencapsulation of mouse islets. Left panel: Incorporation of a fluophore (Alexafluor 680 labelled polylysine) into the multilayers showing complete coverage of the islet surfaces. Right panel: TEM micrograph of nanoencapsulated islets demonstrating normal ultrastructure with an intact nanofilm consisting of nanolayers of thickness around 80 nm covering the outer surface.

In vivo assessment of nanoencapsulated islets in allogeneic transplantation mouse model. To assess whether nanoencapsulation offers effective protection against

immune rejection we have performed preliminary studies using an allogeneic islet transplantation model in which Balb/c islets (H-2d) were transplanted below the kidney capsule of streptozotocin-induced diabetic C57BL/6 mice (H-2b).

The protection from immune assault was confirmed in the study in which we implanted 8-nanolayer coated islets in the allogeneic transplantation model, retrieved graft material at the time about 1 month for histological analysis. As expected, control islets were rejected between 10-14 days after implantation, with the consequent recurrence of hyperglycaemia. In contrast, nanoencapsulated islets maintained normoglycaemia for 28 - 37 days postimplantation (Fig 3) in 5/7 mice, with hyperglycaemia recurring as a consequence of unilateral nephrectomy for graft retrieval for histological analysis rather than immune rejection. Two exceptional mice receiving nanoencapsulated islets reverted to hyperglycemia in less than 28 days due to rejection. Intraperitoneal glucose tolerance test for cured mice showed that the change in glucose level was similar to that of normal nondiabetic mice. Histological analysis of the recovered grafts (Fig 4, left panel) showed no evidence of the infiltration of macrophages into the islets, and sections of kidney showed that significant amounts of islet transplants were not destroyed by host immune attack.

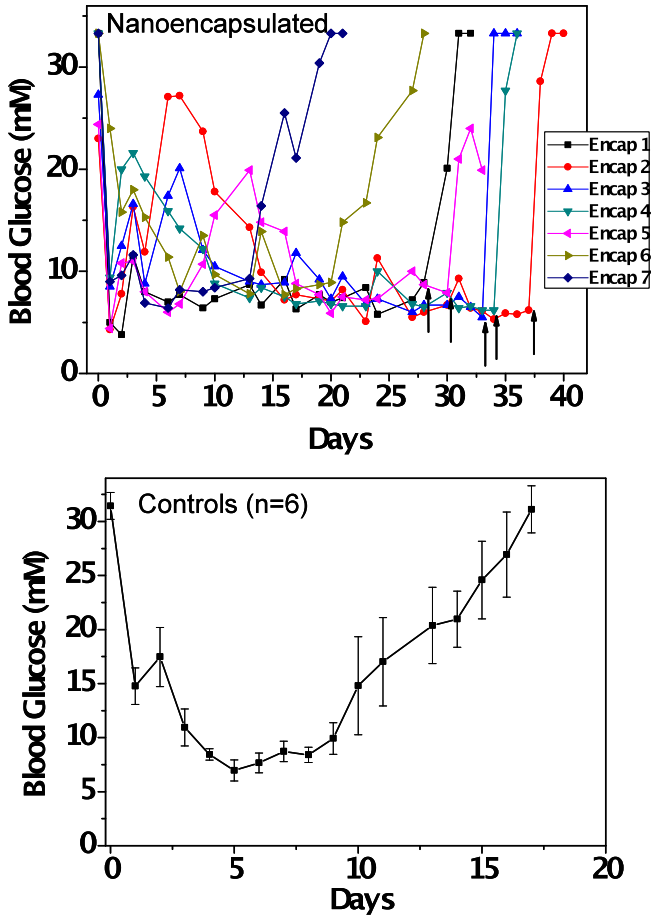

Fig 3. Allogeneic islet transplantation of naked (control) and nanoencapsulated (8 nanolayers) Balb/c islets reversed STZ-induced hyperglycaemia in C57BL/6 mice.

Animals implanted with control, non-encapsulated islets reverted to hyperglycemia (blood glucose >16.7 mM) within 10-14 days (median survival time 12), consistent with graft rejection by the host immune system. Consistent with this, subsequent histological analysis of the nonencapsulated graft material (Fig 4, right panel) revealed extensive lymphocytic infiltration at the subcapsular implantation site, with little or no immunoreactive endocrine material being detectable.

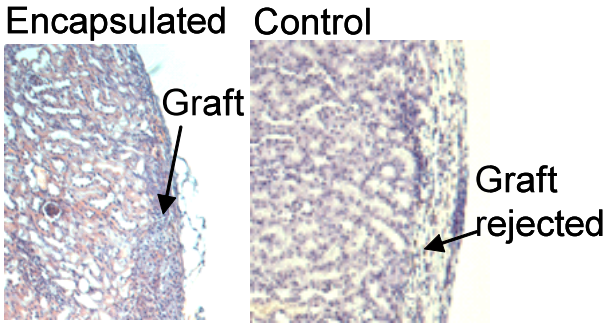

Fig 4. Histological analysis (haematoxylin staining) of recovered graft of nanoencapsulated islets implanted in kidney capsule. Sections of kidney showed that significant amounts of islet transplants were not destroyed by host immune attack compared with the control.

CONCLUSIONS

The nanonanoencapsulated mouse islets were found to preserve appropriate islet secretory function and survival *in vivo*. Allotransplantation studies showed that tailoring the nanoencapsulating nanolayers could optimize islet function post-implantation, allowing complete immunoprotection in mice throughout the one month monitoring period without showing any sign of graft rejection. These results suggest that the nanoencapsulation offers protection for the islets from the host immune system *in vivo*, and may lead to future improvements in clinical islet transplantation which prolong the longevity of islet transplants.

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