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Pseudoislets as primary islet replacements for research

Report on a symposium at King's College London, London UK

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Laboratory-based research aimed at understanding processes regulating insulin secretion and mechanisms underlying β-cell dysfunction and loss in diabetes often makes use of rodents, as these processes are in many respects similar between rats/mice and humans. Indeed, a rough calculation suggests that islets have been isolated from as many as 150,000 rodents to generate the data contained within papers published in 2009 and the first four months of 2010. Rodent use for islet isolation has been mitigated, to a certain extent, by the availability of a variety of insulin-secreting cell lines that are used by researchers world-wide. However, when maintained as monolayers the cell lines do not replicate the robust, sustained secretory responses of primary islets which limits their usefulness as islet surrogates. On the other hand, there have been several reports that configuration of MIN6 β-cells, derived from a mouse insulinoma, as three-dimensional cell clusters termed 'pseudoislets' largely recapitulates the function of primary islet β-cells. The Diabetes Research Group at King's College London has been using the MIN6 pseudoislet model for over a decade and they hosted a symposium on "Pseudoislets as primary islet replacements for research", which was funded by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), in London on 15th and 16th April 2010. This small, focused meeting was conceived as an opportunity to consolidate information on experiences of working with pseudoislets between different UK labs, and to introduce the theory and practice of pseudoislet culture to laboratories working with islets and/or β-cell lines but who do not currently use pseudoislets. This short review summarizes the background to the development of the cell line-derived pseudoislet model, the key messages arising from the symposium and emerging themes for future pseudoislet research.

The Use of Islets for Studies of β-cell Function and Dysfunction

In the late 1960s research on β-cell biology was transformed by the development of techniques for isolating primary islets from whole pancreas by collagenase digestion, followed by a variety of purification strategies.[1-3] These islet preparations enabled detailed studies in controlled in vitro environments that permitted direct measurements of β-cell function rather than indirect measurements in vivo of physiological parameters such as changes in plasma insulin and/or glucose. Over three decades of studies using isolated islets have had a major impact on diabetes research revealing much previously unknown information about the regulation of insulin synthesis, storage and secretion by normal β-cells and of the processes involved in β-cell pathophysiology, including glucotoxicity, lipotoxicity, and responses to inflammatory cytokines. The experimental availability of pure islet preparations also encouraged the development of islet transplantation protocols in animal models of diabetes, which translated into the treatment of Type 1 diabetes in humans by islet transplantation.[4,5] This type of research has required the use of a considerable number of animals as a source of the primary islet preparations.

The PubMed database cites more than 37,000 publications using islets of Langerhans, over 1,500 of which have been published since the start of 2009. For reasons of cost and availability the majority of these studies used rodents (mouse and rat), whose pancreases contain fewer islets than human pancreas (mouse ~200–300, rat ~500–800) but whose islets are of a similar size to human islets.[6] Our estimate, based on our own experience of in vitro islet biology studies, is that each published paper used
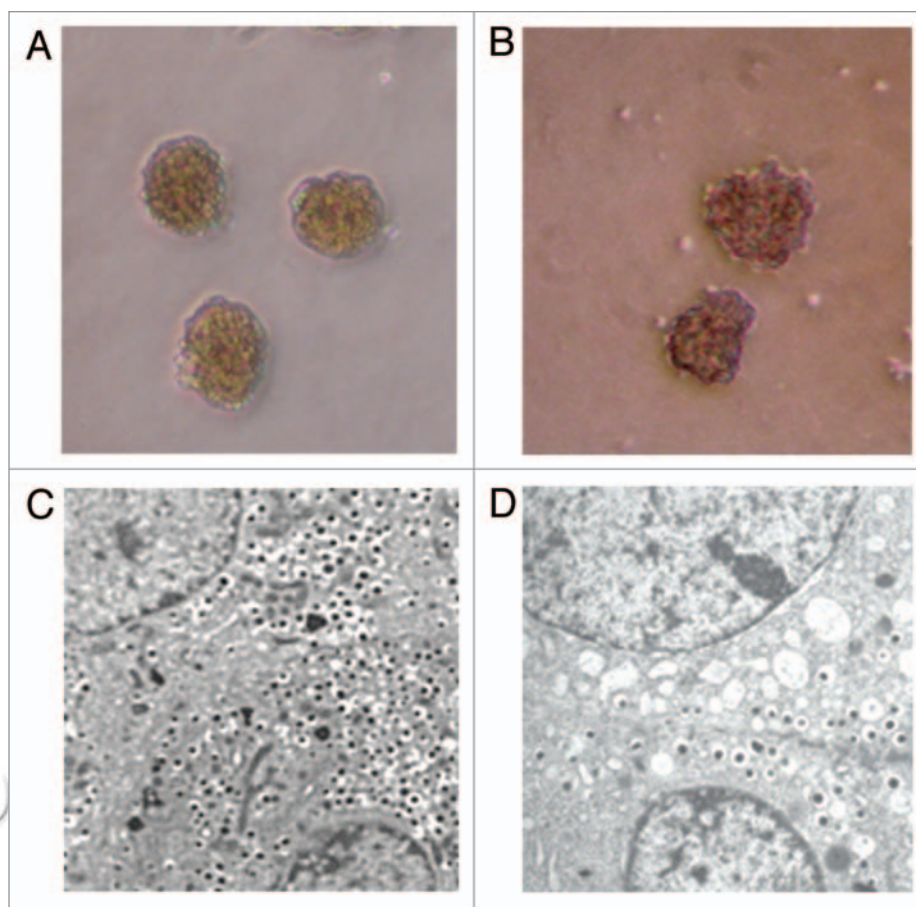
Figure 1. Mouse islets and MIN6 pseudoislets. Primary mouse islets isolated by collagenase digestion (A) are similar in morphology and size (~200 μm) to MIN6 pseudoislets after seven days in culture (B). Ultrastructural analysis shows similar morphologies (islet C, pseudoislet D), although the pseudoislet cells contain fewer dense-cored insulin secretory granules, consistent with the lower insulin content of MIN6 cells compared to primary β-cells.[8]

islets isolated from approximately 100 animals. The data published in 2009/10 alone may therefore have required the use of 150,000 rodents as a source of islets, and this is probably a conservative estimate. In any event, the isolation of primary islets of Langerhans requires the sacrifice of many thousands of laboratory animals each year, so it is timely for research in this area to consider alternatives to rodent islets, where possible.

What Islet Alternatives are Available for Research?

Human islets of Langerhans. Human islets are the obvious "gold standard" model for clinically-relevant studies of islet function and dysfunction, and the success of new islet transplantation regimens has placed the spotlight on human β-cells in the last decade.[4,5] The US diabetes research charity, the Juvenile Diabetes Research Foundation (JDRF), has established a Human Islet Resource Center to supply human islets to NIH- or JDRF-funded laboratories in North America. The JDRF is also funding the European Consortium for Islet Transplantation and one group in the UK to "provide human islets for research projects relevant to our [JDRF's] mission."[7] However, there are a number of obstacles to human islets offering an "off the shelf" alternative to rodent islets in the foreseeable future. First, the supply

of suitable human pancreas for islet isolation is considerably less than the potential demand by transplant recipients with Type 1 diabetes, severely limiting the islet supply. Second, most centers capable of isolating human islets are based around a clinical islet transplantation facility so most of the islets generated are, not surprisingly, prioritized for transplantation rather than research. Third, the high costs associated with isolating human islets in the requisite Good Manufacturing Practice facility adds significantly to the consumable costs of basic research studies, again restricting the widespread use of human islets for research. Finally, the numerous potential sources of variation between pancreas donors (e.g., age, weight, clinical history) and in islet isolation procedures (e.g., cold ischaemia time, collagenase, digestion and culture conditions) makes it inevitable that human islet preparations are considerably more heterogeneous than islets prepared under controlled conditions from genetically uniform, healthy rodents.

Pseudoislets Derived from Hormone-Secreting Cell Lines

A variety of rodent islet hormone-secreting cell lines that can be propagated in culture in vitro are currently available for research purposes (reviewed in ref. 8). These transformed cells

are normally maintained as proliferative monolayers on tissue culture substrates and with increasing passage they tend to lose the differentiated functions of primary islets, such as high levels of hormone expression or biphasic glucose-induced insulin secretory responses. This limits the usefulness of transformed monolayer cell lines in physiologically-relevant studies, which may account for the continued high rate of usage of animals as a source of primary islets. One problem with cell lines is in their usual configuration as adherent monolayers. Thus, it is well established that the anatomical configuration of an islet is important for the maintenance of normal insulin secretory responses (reviewed in ref. 9): dispersing islets into cell suspensions greatly reduces the ability of the component β-cells to respond to physiological signals, and insulin secretion can be restored by reforming the islet cells into three-dimensional islet-like structures which were called pseudoislets in the original studies.[10-12] Consistent with observations made using primary islets, it has subsequently been established that reconfiguring mouse insulin-secreting cell lines (MIN6, βTC6) into three-dimensional structures that closely mimic their native islet architecture (**Fig. 1**) will restore at least some of their differentiated function.[13,14] These β-cell line pseudoislets show much improved insulin secretory characteristics compared to equivalent cells maintained as monolayers, demonstrating the importance of homotypic β-cell to β-cell interactions in the generation and maintenance of appropriate insulin secretory responses, and validating the pseudoislet model for in vitro studies of the regulation of insulin secretion. Since the first publication of the characteristics of MIN6 pseudoislets in 1999,[13] several other laboratories have adopted cell line-derived pseudoislet models for research,[15-19] but this model is still not widely used despite the advantages of widespread availability, simplicity and cost-effectiveness. The Diabetes Research Group at King's College London recently (April 2010) hosted a symposium with the aims of disseminating knowledge about the pseudoislet model and highlighting emerging research themes from current studies using pseudoislets.

Symposium Overview: Pseudoislets as a Substitute for Primary Islets for Research

The symposium offered a mix of review lectures and shorter project-focused presentations to showcase the application of pseudoislet models in on-going research projects in five different research groups across the UK. Astrid Hauge-Evans reviewed the various mechanisms through which islet cells can communicate—cell adhesion molecules, gap junctions and paracrine signaling—and considered these in the context of "why do pseudoislets function better than monolayers?" concluding that it was likely to involve multiple mechanisms. Christina Reers presented data on the functional consequences of inhibiting MIN6 pseudoislet cell proliferation by mitomycin C administration, and suggested that configuring MIN6 cells as pseudoislets increased their resistance to toxic insults. Mustafa Younis described his studies using confocal imaging of MIN6 pseudoislets to define the relationship between pseudoislet formation and the expression of the calcium sensing receptor and of the calcium-dependent cell adhesion molecule E-cadherin (ECAD). The functional consequences of extending

the MIN6 pseudoislet model by incorporating other islet cell lines (αTC1.9 and TGP52 δ-cells) were explored by Catriona Kelly. Her demonstration that the α- and δ-cells localized to the mantle of the heterogeneous pseudoislets suggests that the islet cell lines contain sufficient molecular information to ensure the formation of anatomically-correct islet-like structures. Moira Harrison and Claire Marriott have been exploring different techniques for generating pseudoislets from MIN6 cell populations, and they presented some stunning scanning electron micrographs of pseudoislets created in a microgravity bioreactor, focusing on the significance of the extracellular matrix deposited when MIN6 cells are configured as pseudoislets by this method. The presentation by Mark Russell suggested that anatomically-dependent improved functionality was not a common property of all insulin secreting cell lines. His demonstration that the glucose-responsiveness of rat derived INS-1 cells was not improved when the cells were configured as pseudoislets confirmed previous anecdotal reports using the RINm5F cell line, and may provide another route through which to identify the important underlying mechanisms.

Two separate presentations reported the use of MIN6 pseudoislets as models for islet encapsulation prior to transplantation. Zheng-liang Zhi described using MIN6 pseudoislets to develop novel strategies for nanoencapsulation of islets with multiple layers of charged molecules of defined composition. He presented preliminary data suggesting that this strategy may protect β-cells destined for transplantation, while ensuring that their glucose-induced secretory function is not compromised. Aileen King continued on the transplantation theme by reviewing the effects of transplanted MIN6 monolayers and pseudoislets on blood glucose levels in streptozotocin-induced diabetic mice, and she presented some data using the more conventional alginate encapsulation methodology.

Peter Bergsten extended the pseudoislet network from the UK to Sweden by giving a plenary lecture on "proteomic identification of pseudoislet signalling cascades." Peter's presentation focused on proteins that are upregulated when MIN6 cells are configured as pseudoislets, and elegant pathway analyses indicated that three main groups of proteins showed enhanced expression: those of the ECAD/catenin cascade, enzymes involved in glucose metabolism, and proteins regulating translation. Upregulation of ECAD and glycolytic enzymes fit well with observations made by other groups at the mRNA (arrays) and protein (western blotting) level, providing a high level of consistency from lab to lab in the characterization of MIN6 pseudoislets. Furthermore, the demonstration of upregulation of ribosomal proteins will provide a new focus of investigation in studies aimed at identifying why MIN6 pseudoislets are functionally superior to monolayer cells.

The scientific presentations were complemented by a presentation by Dr. Emma Willoughby, NC3Rs Programme Manager, who provided an overview of the NC3Rs mission, covering successful NC3Rs-funded projects that had led to reduction, replacement or refinement of animal use in research in the UK. She also gave an overview of the current NC3Rs funding schemes, with a quick guide to writing a successful NC3Rs grant application.

Although all of the meeting participants are actively involved in β-cell research, not all had prior experience of generating or

Figure 2. Laboratory workshop on pseudoislet generation. Symposium participants gained experience of pseudoislet generation, handling and functional assays during a laboratory workshop. This hands-on session was aimed at facilitating transfer of pseudoislet protocols to laboratories outside of King's College London.

and its associated intracellular signaling networks in particular, was discussed in the context of a number of the presentations, suggesting that these networks may offer productive targets for future investigations. The mechanisms involved in regulating the balance between cell proliferation, specialized differentiated function and organ formation was another common theme of the discussions, raising interesting questions which may be relevant in the wider context of generating functionally competent islet substitutes for transplantation therapy of diabetes, such as cells generated from stem cell populations. The value of preliminary screening using "-omic" technologies to identify new targets for investigation was discussed in the context of the encouraging correlations between results from genomic and proteomic screens. The implication from proteomic/genomic studies is that we may now need to harness the power of metabolomic technologies to understand why pseudoislets are functionally superior to their constituent cells in isolation.

In summary, the consensus view was that pseudoislets offer a simple, cost-effective and valid replacement for primary islets in certain areas of islet biology research. However, it was recognized that rodent islets still have an important role in reproducing key observations made in pseudoislets, as models of human islets in transplantation studies, and for studies of regulation of the β-cell mass in which transformed cells are a poor model. Readers of this report who are interested in learning more about the MIN6 pseudoislet model, or in transferring the methodology to their research programmes should contact the corresponding author (shanta.persaud@kcl.ac.uk).

using pseudoislets so hands-on laboratory demonstrations were provided by Astrid Hauge-Evans, Christina Reers and Aileen King (**Fig. 2**). The focus of these was to provide familiarity with pseudoislet formation over a week in culture; methods of pseudoislet fixation and processing for immunocytochemistry; assessment of pseudoislet viability and dynamic secretory function; and encapsulation of pseudoislets for transplantation studies. Participants in the laboratory sessions obtained hands-on experience of handling pseudoislets to facilitate transfer of the methodology and expertise back to their host laboratories.

Emerging Themes

Limiting the size of the symposium encouraged friendly, open and extensive discussions from which a few key themes emerged. The role of cell adhesion molecules in general, and of ECAD

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