INDUCED CELL CLUSTERING ENHANCES ISLET β CELL FORMATION FROM PANCREATIC DUCTAL EPITHELIAL CELLS

Michael I. Boretti, Keith J. Gooch

Department of Bioengineering
University of Pennsylvania
Philadelphia, PA 19104

ABSTRACT

Although islet transplantation holds great promise as a treatment for diabetes, a major limitation to its future clinical use is a shortage of donor tissue. During both fetal development and adult pancreatic regeneration, pancreatic ductal epithelial cells (PDEC) differentiate into endocrine cells. The goal of this project is to apply existing understanding of these processes to stimulate islet cell differentiation in vitro. Following 23-24 days of culturing adult human PDEC in a standard cell culture environment (tissue culture treated plastic, 10% FBS), they transdifferentiate to β cells at an extremely low efficiency (0.115 +/- 0.007%). β-cell differentiation was enhanced 4-fold (0.46 +/- 0.03%) when PDEC were grown on a low adhesive substrate (nontreated plastic) and in a low serum medium (5% Nu serum), conditions which are permissive to cell detachment and clustering. While the overall efficiency of transdifferentiation was still very low, the results suggest that induced cell-cell interactions and/or cell shape changes may be critical to stimulating β–cell production in vitro. We are currently exploring additional factors (ECM factors, growth factors, transcription factors) that may improve the efficiency of transdifferentiation in order to optimize the creation of tissue-engineered islets.

Keywords - diabetes, tissue-engineering, transdifferentiation, islets

INTRODUCTION

Type I diabetes is an autoimmune disorder which results in the destruction of pancreatic islet β cells and the ensuing loss of their secretory product insulin, a key regulator of glucose homeostasis. The standard treatment for this disease is insulin injection, but the intermittent nature of this therapy makes the tight control of blood glucose impossible to achieve, often resulting in hypoglycemic attacks and long-term complications such as cardiovascular disease, kidney failure, neuropathy, and blindness. Recently, the injection of donor allogenic islets has been shown to cure diabetes in clinical trials [1]. Although this research has lead to new hope for diabetics, a major barrier to its widespread utility is a scarcity of donor tissue, as there are only enough donor islets available to treat a very low percentage of the diabetic population.

Although several approaches have been investigated in an effort to generate large amounts of cells capable of secreting insulin in response to glucose, perhaps the most attractive method is to tissue-engineer islets by recapitulating islet neogenesis, a normal process that occurs during both fetal development and adult pancreatic regeneration by which pancreatic ductal epithelial cells (PDEC) transdifferentiate into islet tissue. While other groups have demonstrated the feasibility of transdifferentiating PDEC to islet cells in vitro, yield has been far too low to be clinically useful [2,3]. The goal of this work is to optimize the tissue-engineering of islet β cells through controlled cell clustering.

MATERIALS AND METHODS

Isolation of and culture of PDEC

Human islet isolations were performed by the Human Islet Isolation Core Facility at the University of Pennsylvania using the Ricordi Method [4]. After centrifugation through a discontinuous Ficoll gradient, the layer of tissue below the islet layer, enriched with ductal fragments and exocrine tissue, was collected (<1% islets). Tissue from this layer was suspended in CMRL + 10% FBS to inactivate residual collagenase, washed three times in HBSS + 5 mM glucose + 50 μM $CaCl_2$, resuspended in CMRL + 10% FBS, and plated on either tissue culture treated plastic (TCP) at a density of 20-25 μL cell pellet/6-well plate or non-treated tissue culture plastic (NTP) at a density of 40-45 μL cell pellet/6-well plate. After 24 hours and 48 hours, respectively, non-adherent tissue was removed from both the TCP and NTP plates, and cultures were re-fed with fresh CMRL + 10% FBS. After 1 week of culture, medium from half of the cultures was changed to SFDM + 5% Nu-Serum IV, a well-defined, low-serum medium for ductal epithelial cell growth. Culture medium was replaced every 2-3 days.

Phenotypic Analysis

The ductal phenotype of the monolayer cultures was confirmed with immunocytochemistry for cytokeratin 19, a well-established PDEC marker. Insulin expression was quantified using diphenylthiocarbazone (DTZ) staining and insulin immunohistochemistry (IHC). DTZ is a zinc chelator that quickly and specifically stains β cells red and was directly applied to cell cultures for analysis. Insulin IHC was performed on paraffin sections of PDEC embedded in agarose using a 1^{o} guinea pig anti-human insulin mAb and a 2^{o} FitC-conjugated anti-guinnea pig Ab. Following DTZ and IHC staining, the percentage of β cells was determined by randomized cell counts. Data was accumulated from experiments from 2 separate isolations.

RESULTS

Immediately following isolation, the fraction of islet β cells in the duct/acinar-enriched layer of pancreatic digest was 0.4%. By day 3 of culture, the initial adherent tissue had begun to spread and form a monolayer. At this time, no DTZ+ cells were detected (Figure 1) as islet tissue has been demonstrated to attach to plastic much less readily than ductal and acinar tissue [2]. By day 7, the cells had spread to form a monolayer with the majority of cells expressing the ductal marker CK19, but still no DTZ+ cells were detected in any of the cultures. At this time the medium from half of the cultures was changed to SFDM + 5% Nu, with the other half of the cultures remaining to be fed with CMRL + 10% FBS.

Relatively soon after addition of SFDM + 5% Nu (within 24 hours), PDEC grown on NTP appeared to lose their spread morphology and tight attachment to the substrate and take on a more rounded shape. During the next 2 weeks, PDEC gradually detached from the substrate (especially at the edges of the epithelial colonies) and formed cell aggregates that morphologically resembled islets. Although some β cells were observed in the monolayer regions, they were preferentially located within the cell aggregates. Although less pronounced, cell clustering also occurred in cultures of PDEC *either* grown on NTP *or* fed with SFDM + 5% Nu Serum, but was not observed in cultures grown on TCP and fed with CMRL + 10% FBS.

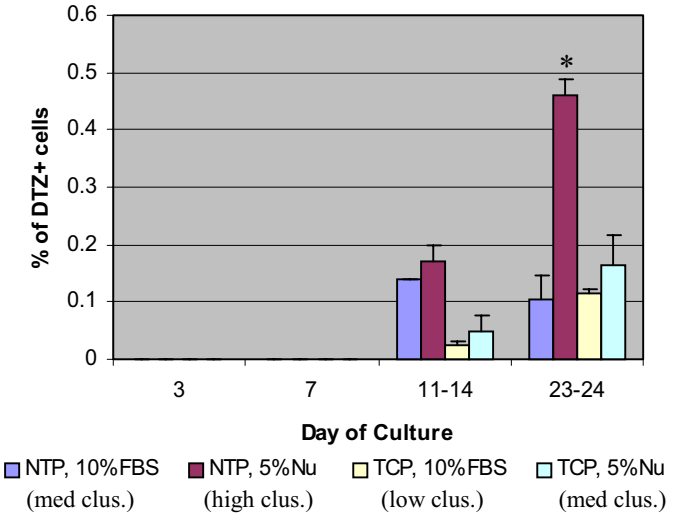

Figure 1: Percentage of dithizone-stained cells as a function of time and culture environment (degree of cell clustering is listed in parentheses)

Phenotypic analysis between days 11-14 revealed the presence of a low percentage of DTZ+ cells under all conditions tested (<0.2%). DTZ staining between days 23-24 revealed a further increase in the percentage of DTZ+ cells, with a significantly higher percentage in those cultures grown on NTP with 5% Nu Serum (0.46 +/- 0.03%) than under all other conditions tested (Figure 1). Insulin immunohistochemistry was done on samples of parallel cultures from day 24, and the percentage of insulin+ cells was demonstrated to correlate very well with the percentage of DTZ+ cells (Figure 2).

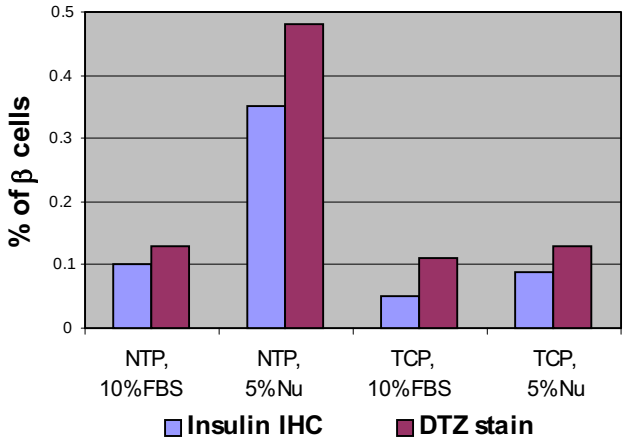

Figure 2: Percentage of β cells at day 24 for a particular sample as calculated by insulin IHC and DTZ staining

DISCUSSION

In this study, islet β cells were produced at a very low efficiency from a culture of normal PDEC grown on TCP, as determined by dithizone and insulin staining. Simple modification of the culture conditions to include a non-adhesive substrate and Nu serum, a serum substitute reduced in attachment factors, resulted in cell clustering and a significant enhancement of β–cell production. While this improvement still does not come close to addressing the clinical shortage of donor tissue, it provides insight into a potentially important role of cell shape changes and cell-cell interactions in the transdifferentiation process. Currently, we are using microarrays and RT-PCR to study the effects of various modifications in culture environment on PDEC transdifferentiation to islet cells. By better understanding the molecular effects (upstream of overt diffferentiation) of differentiation stimuli, we hope to better design protocols for the tissue engineering of islets.

REFERENCES

1. Shapiro AM, et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *N Engl J Med*, 2000, Jul 27;343(4):230-8.
2. Bonner-Weir S, et al., "In vitro cultivation of human islets from expanded ductal tissue," *Proc Natl Acad Sci USA,* 2000, Jul 5;97(14):7999-8004.
3. Kerr-Conte J, et al., "Ductal cyst formation in collagen-embedded adult human islet preparations. A means to the reproduction of nesidioblastosis in vitro," *Diabetes*, 1996, Aug;45(8):1108-14.
4. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW, "Automated method for isolation of human pancreatic islets", *Diabetes*, 1988, Apr;37(4):413-20.